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DEMONSTRATION OF REAGIN OF A NEW SPECIFICITY IN SERA OF TREATED RAGWEED-SENSITIVE PERSONS

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IN 1911, Noon[1] demonstrated that patients suffering from hay fever showed clinical improvement after they had received a series of graded injections of pollen extract. This procedure of "desensitization" or "hyposensitization" has since been modified only slightly and constitutes the main treatment offered to clinically allergic persons. The mechanism by which protection is afforded by the injection of the pollen extract is still poorly understood. In 1935 Cooke and associates[2] demonstrated the presence of a factor in sera of treated allergic persons not found in sera of nontreated persons. This factor, which they referred to as "blocking antibody," was capable of combining with the allergen in vitro and of preventing it from reacting in vivo with the skin-sensitizing factor or reagin. The presence of blocking antibody in treated allergic persons was later confirmed by Loveless,[3] Frank and Gelfand,[4] Sherman,[5] Cooke and colleagues,[6] and more recently in this laboratory.[12] Although Loveless[7] claims that this is the factor responsible for the clinical improvement shown in treated patients suffering from hay fever, other attempts at correlating blocking antibody titers with clinical results have not been very rewarding.[5, 8-11]

The present study was undertaken to demonstrate differences which may exist between sera of treated† and nontreated allergic persons with respect to their reactivity to fractions of ragweed pollen extract.

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†All ragweed-sensitive persons who had undergone at least one series of injections of ragweed pollen extract are referred to as treated or desensitized. Sera obtained from such persons are referred to as treated sera as compared to untreated sera from untreated allergic persons.

METHODS AND RESULTS

Two allergen preparations of ragweed pollen *(Ambrosia artemisiaefolia)* were used: (1) WSR, the whole water-soluble extract of defatted ragweed pollen and (2) dialysate, the fraction of WSR which dialyzed through Visking

TABLE I. REAGINIC ACTIVITY IN SERA AND SKIN OF UNTREATED ALLERGIC INDIVIDUALS TO DIALYSATE AND WSR

SERUM OR SKIN OF PATIENT TESTED	REACTION BY PASSIVE TRANSFER TO:		REACTION BY DIRECT SKIN TEST TO:	
	DIALYSATE	WSR	DIALYSATE	WSR
B. P.	0	5+	*	*
A. L.	0	5+	*	*
A. P.	0	5+	*	*
L. M.	0	2+	0	4+
M. G.	0	3+	0	6+
D. G.	0	4+	0	6+
S. R.	0	4+	0	4+
P. G.	0	4+	0	6+
A. W.	0	1+	0	3+
#9	0	3+	*	*
#10	0	3+	0	2+
#11	0	2+	0	2+
#18	0	5+	*	*
#22	0	2+	*	*
#23	0	3+	*	*
#48	0	2+	*	*
#49	0	1+	0	3+
#50	0	3+	*	*
#51	0	3+	0	3+
#53	0	4+	1+	4+
#54	0	4+	1+	5+
#57	0	4+	4+	5+
#58	0	4+	*	*
#59	0	4+	*	*
#62	0	4+	1+	4+
#64	0	2+	*	*
#65	0	2+	*	*
#66	0	2+	1+	3+
#68	0	4+	*	*
#69	0	3+	0	5+
H. L.	1+	5+	*	*
R. A.	2+	2+	*	*
#52	3+	3+	2+	4+
#61	2+	2+	*	*
#63	3+	2+	2 +	4+
#67	2+	4+	*	*
P. M.	*	*	0	5+
R. N.	*	*	1+	4+
R. S.	*	*	0	6+
R. K.	*	*	0	4+
F. J.	*	*	0	2+
#60	*	*	2+	4+
#71	*	*	6+	6+
#73	*	*	3+	6+
#74	*	*	0	2+

*Test not performed.

cellophane tubing in twenty-four hours at 4° C. Both allergen preparations were dissolved in saline and prepared in concentrations of 1,000 units per milliliter (1 unit = 10^{-5} mg. nitrogen).

Blood was drawn from treated and untreated ragweed-sensitive patients and the sera were separated by centrifugation and sterilized by Seitz

filtration. The undiluted serum (0.05 ml.) was injected into two sites on the back of a normal, nonallergic person; twenty-four hours later the sensitized sites were challenged with WSR and dialysate and the reactions were graded 0, 1+, 2+, 3+, etc., depending on the size of the wheal and the surrounding erythema.

As can be seen from Tables I and II, when the method of passive transfer was used, all sera reacted to WSR. On the other hand, only six of thirty-six sera from untreated and twenty-seven of thirty-two sera from treated subjects reacted to dialysate.

Intradermal skin tests were performed on untreated and treated persons with both WSR and dialysate. Only eleven of twenty-six untreated persons, as compared to all of the ten treated persons, reacted to dialysate. All patients reacted to WSR (Tables I and II).

TABLE II. REAGINIC ACTIVITY IN SERA OF TREATED ALLERGIC PERSONS TO DIALYSATE AND WSR

SERUM TESTED	REACTION BY PASSIVE TRANSFER TO:	
	DIALYSATE	WSR
B. P.	5+	5+
F. W.	4+	4+
A. S.	3+	3+
R. D.	5+	4+
#3	2+	2+
#4	1+	4+
#5	1+	3+
#7	3+	4+
#10	1+	4+
#11	1+	2+
#12	2+	3+
#14	4+	4+
#13	1+	1+
#25	4+	4+
#28	2+	3+
#29	2+	3+
#35	4+	4+
#36	2+	3+
#37	1+	2+
#38	4+	4+
#40	3+	5+
#41	2+	3+
#42	4+	3+
#43	4+	4+
#45	4+	4+
#55	3+	3+
#56	2+	4+
#22	0	3+
#24	0	3+
#33	0	3+
#44	0	2+
#46	0	4+

In addition, ten persons who had undergone treatment reacted without exception by direct skin test to dialysate and WSR. All reactions to dialysate were comparable to reactions evoked by the injection of WSR (3+ or greater).

In addition, sera were obtained from five allergic patients before and after they had undergone desensitization treatment. Both the pre- and post-treatment sera were tested by passive transfer. As can be seen from Table III, none of the five sera had reagins to dialysate before treatment. After treatment, however, the sera of four of these five persons possessed reagins to

dialysate demonstrable by passive transfer. The fifth patient became reactive to intradermal injection of dialysate, although no reagin directed toward dialysate could be detected in his serum by the passive transfer method. Sera obtained from one of the five patients (B.P.) one month and eighteen months after his last ragweed injection were equally reactive to dialysate (Table III).

TABLE III. REAGINIC ACTIVITY IN SERA OF SENSITIVE PERSONS BEFORE AND AFTER TREATMENT

SERUM TESTED	REACTION BY PASSIVE TRANSFER TO:	
	DIALYSATE	WSR
B. P. Pre*	0	5+
Post†	5+	5+
Post 2‡	5+	5+
#10 Pre	0	3+
Post	1+	4+
#11 Pre	0	2+
Post	1+	2+
#18 Pre	0	5+
Post	2+	4+
#22§ Pre	0	2+
Post	0	3+

*"Pre" refers to serum obtained before treatment.

†"Post" refers to serum obtained from the treated person after the ragweed season.

‡This serum was taken eighteen months after Patient B. P. received his last injection of ragweed pollen.

§Patient #22 reacted by skin test to dialysate (2+) after undergoing treatment, although his serum contained no detectable reagins by passive transfer.

Two sera containing blocking antibodies were tested for their capacity to inactivate the allergens in the dialysate by the method used in this laboratory.[12] Neither of these sera demonstrated any ability to inactivate dialysate in vitro although both, in serum dilutions as high as 1:32, were capable of blocking WSR.

DISCUSSION

Since 86 per cent of all treated sera and only 17 per cent of all untreated sera possessed reagins which reacted to dialysate, it appears that reagins of a new specificity were formed by ragweed-sensitive patients undergoing desensitization treatment with ragweed extracts. In fact, the presence of these reagins after treatment and their absence prior to treatment was demonstrated in the sera of four out of five patients (Table III).

The significance of the reagins detected only in the sera of treated patients is not clear at this time. It is possible that they may be responsible for the increased clinical sensitivity observed in some treated persons. Patients B. P. and B. W. both claimed to have had more pronounced clinical symptoms after treatment, although their sera had blocking antibody titers to WSR of 1:8 and 1:16, respectively. Both possessed reagins to dialysate in high titer in their sera. On the other hand, it is worth mentioning that we have detected reagins to WSR in the skin of many persons who have never

had symptoms of clinical allergy. We have also recently demonstrated[14] that formation of reagins can be induced in a few nonallergic persons without invoking a condition of clinical allergy.

From the foregoing results, it would appear that the "true allergens" (those which induce clinical allergy in untreated persons) are present only in the nondialyzable fraction of WSR, which constitutes only 2 to 4 per cent (by weight) of the whole WSR. With a number of sera from untreated subjects the dialyzed residue of WSR* was substituted for whole WSR in the passive transfer test and results identical with those shown in Table I were obtained.

The mechanism by which both clinical sensitivity and skin-sensitizing activity are induced in susceptible subjects is not known. An "incubation" period of a year or two is generally necessary in the majority of patients coming to a ragweed-laden area for the first time. Presumably, the first exposure to ragweed induces the formation of reagin, and clinical sensitivity may or may not become manifest. In this connection, it is difficult to explain why most allergic persons possessed reagins to the allergens of the dialysate only after treatment. In further studies on this problem three normal, nonallergic volunteers were injected twice a week for six weeks with a large quantity of WSR (a total of 2,500,000 units each). No reagins could be demonstrated, either by direct intradermal test or by the method of passive transfer.

Another observation which requires explanation is why some untreated ragweed-sensitive persons possessed reagins to the allergens in the dialysate. It may be significant that all of these untreated persons who reacted by skin test to dialysate also reacted to some allergens other than ragweed (dust, horse dander, grass, etc.). Since it has been demonstrated previously that cross-reactivity exists between different allergens, it is possible that the reagins reacting with the allergens in the dialysate in these patients were formed on stimulation with other allergens. However, one cannot exclude the possibility that these persons were inherently capable of forming these reagins after exposure to all the allergens in ragweed pollen during the ragweed season.

As can be seen in Table I, five untreated persons gave positive reactions on intradermal skin tests with dialysate, although their sera possessed no reagins to this allergen preparation by passive transfer. Four of these five persons showed small reactions by direct skin test (+1). Therefore, one can conclude that reagins to dialysate were present in negligible quantities in their skins. Two possible explanations for this observation are: (1) The intradermal skin test is more sensitive than the passive transfer test for the detection of small quantities of reagin. This explanation alone, however, seems doubtful in this case, since reactions in these persons by direct skin tests with WSR were not significantly larger than the reactions obtained with their sera by passive transfer. (2) These persons may have formed reagins

*The fraction of WSR left inside the dialyzing sac after twenty-one days of dialysis against running tap water is referred to as the dialyzed residue of WSR.

in very low concentration to the allergens in the dialysate during the ragweed season. These reagins may have been "bound"* to the skin and remained there while their concentration in the blood diminished when further stimulation for their formation ceased.

CONCLUSIONS

1. It has been shown that the injection of whole ragweed pollen extract into ragweed-sensitive subjects leads to the formation of reagins not present before treatment.

2. These reagins have a different specificity as compared to the "normal" skin-sensitizing antibody formed in untreated allergic persons.

We would like to thank Mr. Tom Webb for fruitful discussions.

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*Loveless,[13] in 1941, showed that reagin has a very marked affinity for the skin and is capable of being "bound" to it.

THE USE OF COLOR-CODED TABLETS IN THE CLINICAL APPRAISAL OF TWO ESTABLISHED BRONCHODILATOR PREPARATIONS AND N-[β-(10-PHENOTHIAZINYL)PROPYL] TRIMETHYL-AMMONIUM BENZENE CHLORIDE

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THE appraisal of new drugs frequently becomes the responsibility of the physician. In view of the discrepancies between experiments in animals and experience in human patients, only extensive clinical trials will determine whether or not a new drug has the effects that preliminary experiments may have suggested. In the case of oral preparations, the usual procedure is to compare the new compound with one or more drugs of known activity and with a placebo. The pharmacologic agents, identical in appearance and unidentifiable by both physician and test subject (double-blind technique), are then dispensed in a randomized sequence to suitable subjects and the subjective response to each is carefully noted and assessed. Such an investigation is time consuming and rather cumbersome, and it often takes many months to complete it. In the hope of facilitating the procedure, an attempt was made to obtain similar information by the use of preparations coded by color and dispensed together in a single bottle.

MATERIALS* AND METHODS

In the period extending from October, 1955, to November, 1957, sixty-three patients with asthma, nine of whom had an irreversible obstructive component to their pulmonary disease (emphysema), served as subjects for this study. All of the patients were ambulatory. There were twenty-six male and thirty-seven female patients, with a median age of 39 for males and 41 for females; the median duration of the disease was ten years for both sexes. On the basis of duration, frequency, need of medication, and tests of pulmonary function, all patients had mild to moderate symptoms of obstructive pulmonary disease at the time they were included in the study. Forty patients were receiving injections of allergenic extracts and continued to do so; eight patients had been on one or another of the corticosteroid drugs and, during the period of the investigation, received one or more additional courses of steroids.

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In addition to the placebo, the drugs used in this study were Hyadrine,* aminophylline with racephedrine† (drugs of known bronchodilator activity), and a new drug—N-[β-(10-phenothiazinyl)propyl] trimethylammonium benzene chloride (Sc-7188‡). This drug is a synthetic quaternary ammonium derivative of phenothiazine which in the experimental animal had shown potent antihistaminic, anticholinergic, and suggestive bronchodilator properties.[2] Its chemical structure is shown in Fig. 1. The medications were dispensed according to

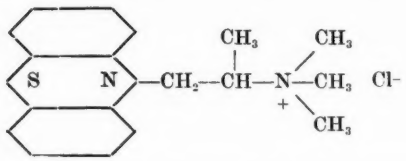

Fig. 1.—Sc-7188, N-[β-(10-phenothiazinyl)propyl] trimethylammonium benzene chloride.

two plans, both "double-blind." According to the first method, each of these four unknowns, identical in appearance and packaged in similar-appearing bottles coded by number, was given in sequence for a period of one week or more, and the subjective response to each preparation was noted. The drugs were dispensed in rotation, but the order was varied. All subjects had a trial with each of the four preparations. The dosage was the same for the four medications, namely, one tablet every three or four hours as required. Upon completion of that portion of the study, the same patients received the same preparations according to the second method. Here the preparations were identified by color§ and dispensed in a single bottle with appropriate instructions as to the manner in which they were to be taken. All patients were able easily to distinguish the four different colors. Briefly, the instructions given the patients were as follows: "When symptoms occur, take any one of the colored tablets; if relief follows, continue taking the tablets of the color selected every three or four hours as required until none remains. On the other hand, if relief is not satisfactory, take a tablet of another color. Continue in this manner until all four preparations have been tried."

In both methods I and II, patients were usually seen at seven- to fourteen-day intervals over a period of many weeks. On the basis of the patient's comments and written notes and the need for ancillary medication, the following criteria were used in scoring the efficacy of the medication: *excellent relief,* if complete or almost complete relief of symptoms occurred within an hour and lasted for three or more hours; *satisfactory relief,* if relief was 50 to 75 per

*Diphenhydramine, 37.5 mg., aminophylline, 150 mg., and racephedrine, 25 mg.

†Aminophylline, 150 mg., and racephedrine, 25 mg.

‡Sc-7188, 60 mg.

§When the investigation was completed and the data were tabulated, the code was broken and the colored tablets were found to be the following:

Blue-green—Lactose (placebo)
Peach—Sc-7188
Yellow—Aminophylline and racephedrine
White—Hyadrine

cent and lasted two or more hours; *fair relief,* if relief was between 25 and 50 per cent and lasted for a minimum of one to two hours; and *no relief,* if relief was less than 25 per cent or if effectiveness was short.

TABLE I. MEAN EFFECTIVENESS RATINGS BY SEX, METHOD, AND DRUG

SEX	METHOD	AMINOPHYLLINE AND RACEPHEDRINE	PLACEBO	HYADRINE	SC-7188	TOTAL
Males (26)	I	1.81	0.69	1.88	1.15	1.38
	II	2.04	0.35	2.42	0.58	1.35
	Both	1.92	0.52	2.15	0.87	1.37
Females (37)	I	2.08	0.65	2.27	1.24	1.56
	II	1.97	0.62	2.35	1.08	1.51
	Both	2.03	0.64	2.31	1.16	1.53
Both sexes (63)	I	1.97	0.67	2.11	1.21	1.49
	II	2.00	0.51	2.38	0.87	1.44
	Both	1.98	0.59	2.25	1.04	1.46

RESULTS

The mean effectiveness ratings for the four preparations under the two methods of administration (measured on a scale of 0-3, increasing with judged effectiveness) are tabulated in Table I. Inspection of this table shows that

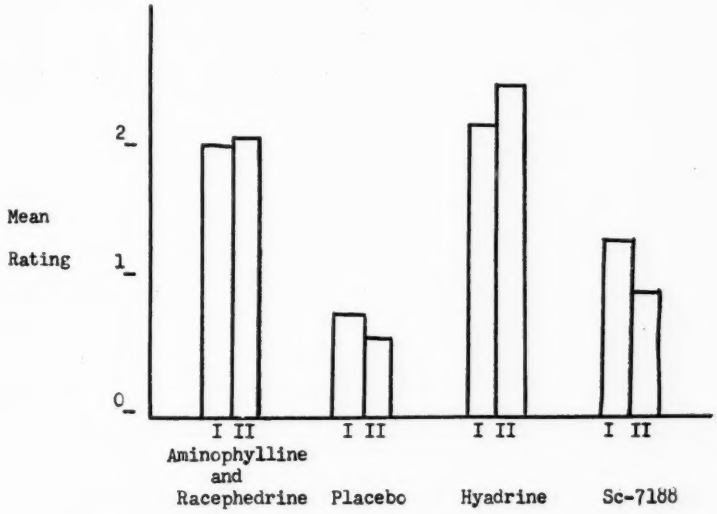

Fig. 2.—Mean ratings by methods with sexes pooled.

there are mean differences among the four preparations, with Hyadrine achieving the highest mean rating, aminophylline with racephedrine next, Sc-7188 considerably lower, and the placebo the lowest. The mean ratings are in the same rank order for both sexes by both methods of administration. Fig. 2 graphically portrays these mean differences, using means for the pooled sexes.

The analysis of variance (Table II) shows that differences among the drug means are highly significant, and there are no other significant effects. The analysis, however, does not tell which differences among pairs of drugs are important. For this, we have used Duncan's[3] procedure as modified by Kramer.[4]

TABLE II. ANALYSIS OF VARIANCE OF MEAN EFFECTIVENESS RATINGS BY SEX, METHOD, AND DRUG

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F
Sex	3.47	1	3.47	2.70
Methods	0.29	1	0.29	<1
Drugs	230.68	3	76.89	85.3**
Sex by method	0.00	1	0.00	<1
Sex by drug	0.71	3	0.24	<1
Drug by method	6.33	3	2.11	2.34
Sex by drug by method	4.57	3	1.52	1.69
Subjects	78.39	61	1.285	1.43
Residual	384.92	427	0.90145	
Total	709.36	503		

**p 0.01.

This test shows that the difference between aminophylline with racephedrine and Hyadrine is not significant, but that Sc-7188 is significantly lower than these two preparations. The placebo is significantly lower than the other three.

TABLE III. FREQUENCY OF COLOR CHOICE FOR METHOD II, ALL SUBJECTS

COLOR COMBINATION	NO. OF TIMES CHOSEN	NO. OF TIMES PAIR OF COLORS CHOSEN AS FIRST-SECOND CHOICE	NO. OF TIMES EACH COLOR CHOSEN FIRST
W Y B P	6		
W Y P B	4	10	
W B Y P	1		
W B P Y	1	2	
W P B Y	1		
W P Y B	1	2	14
Y W B P	1		
Y W P B	2	3	
Y B W P	1		
Y B P W	1	2	
Y P B W	2		
Y P W B	2	4	9
B Y P W	3		
B Y W P	1	4	
B P W Y	4		
B P Y W	10	14	
B W Y P	3		
B W P Y	1	4	22
P B W Y	5	12	
P B Y W	7		
P W B Y	5		
P W Y B		5	
P Y W B	1		
P Y B W		1	18

W = White (Hyadrine).
Y = Yellow (aminophylline and racephedrine).
B = Blue-green (placebo).
P = Peach (Sc-7188).

Since the subjects had free choice of drug by color under method II, a tabulation was made of the number of times that each of twenty-four possible color orders was chosen, and this is shown in Table III. In the first column of figures may be seen the number of times that each combination was chosen, and the second column shows the number of times that a pair of colors was picked as the first-second choice. The third column shows the number of times that each of the four colors was selected first. Although the blue- and peach-colored tablets were selected first more than the other two, this is not conclusive evidence of color bias. Considering first choice only, a purely random procedure of selection would assign about sixteen choices to each color. Departures from this pattern can be tested for significance, using a chi-square test with three degrees of freedom. This test yields a chi-square value of 5.89, with a corresponding probability value greater than 0.10, a nonsignificant result. There is some evidence of a bias toward the blue-peach or peach-blue combination, but we doubt if this is important.

SIDE EFFECTS

A tabulation of reported side effects showed that thirty-two of the sixty-three patients given the four preparations reported a total of seventy-six effects. Of these untoward reactions, forty-two were found in method I and thirty-four in method II. Effects by type and drug are shown in Table IV. Of the total

TABLE IV. SIDE EFFECTS PRODUCED IN THIRTY-TWO OF THE SIXTY-THREE PATIENTS, BY TYPE, DRUG, AND METHOD

	DRUG AND METHOD								
	HYADRINE		AMINOPHYLLINE RACEPHEDRINE		SC-7188		PLACEBO		
TYPE	I	II	I	II	I	II	I	II	TOTAL
Nervousness	6 (6)*	7	11 (10)	11	1			2	38
Drowsiness	6 (4)	3			3 (2)	1	2 (4)	5	20
Nausea	1		4 (4)	2	5 (6)	3	1		16
Headache	2								2
Total of all effects	15	10	15	13	9	4	3	7	76

*The distribution of paired effects is shown by the numbers in parentheses.

number of side effects, fifty-three were directed toward Hyadrine and aminophylline with racephedrine with the remaining effects divided almost equally between the other two preparations. In order of decreasing incidence, the side reactions seen were nervousness, drowsiness, nausea, and headache.

When the side effects were viewed by methods, thirty-six of the seventy-six reported effects (47 per cent) were "paired," that is, the same symptom was described by the same subject for the identical preparation under both methods (Table IV). The paired effects were due to fifteen patients, three of whom

accounted for twelve of the thirty-six paired responses. Of these three patients (each of whom had two paired side effects), one reported drowsiness while taking Hyadrine and nervousness following aminophylline with racephedrine. Another stated that he had nausea from both Sc-7188 and aminophylline with racephedrine. The third patient developed drowsiness from Hyadrine and nausea from aminophylline with racephedrine.

DISCUSSION

The data presented here indicate that the patients derived the most benefit from the known bronchodilator compounds, Hyadrine and aminophylline with racephedrine. The placebo was the least effective, and Sc-7188 occupied a disappointing intermediate place, with respect to effectiveness, between the placebo and the other two.

Judging from the lack of significant method differences and method-by-drug interaction, it appears that the two methods give equivalent results in so far as drug evaluation by mean ratings is concerned. This, of course, does not necessarily imply that subjects give equivalent ratings to a drug under the two methods of administration. It merely means that, subject by subject, differences tend to be self-canceling, that is, there is no apparent bias attributable to the method of administration.

TABLE V. FREQUENCY OF RESPONSE RATINGS ON ALL DRUGS BY TWO METHODS

		METHOD II				
		0	1	2	3	
METHOD I	0	46	13	9	9	77
	1	11	12	13	8	44
	2	14	12	20	18	64
	3	9	7	23	28	67
		80	44	65	63	252

To investigate this aspect further, all ratings on all drugs were cross-tabulated by method, as shown in Table V. The total of the frequencies in the main diagonal of Table V (upper left to lower right) gives the number of pairs of ratings in complete agreement for the two methods. There were only 106 such ratings out of the total of 252 pairs of ratings, or 42 per cent. Since the two methods give quite similar results, one might expect a higher correlation. However, considering that the source of the rating is the investigator's evaluation of the patient's statement as to his reaction to the drug, we feel justified in defining a difference of no more than one scale point as constituting "close agreement." Under this definition there are 196 pairs out of 252, or 78 per cent, a close correlation indicative of a considerable degree of consistency between the two methods. The fifty-six remaining pairs were distributed among the various drugs as follows: aminophylline with racephedrine, twelve; placebo, eleven; Hyadrine, fourteen; and Sc-7188, nineteen. Since considerable time elapsed between the two administrations, lack of agreement can be a function

of changes in the subject as well as of method. The extent to which changes in the patient are responsible for disagreements among ratings, of course, cannot be inferred from the data.

The side effects induced by the four preparations in thirty-two of the sixty-three patients were mild to moderate in severity and, in general, were unimportant (Table IV). Although the incidence of effects varied somewhat by method, this was not considered significant and, for purposes of presentation, the effects seen in both methods will be discussed together. Although aminophylline with racephedrine did not engender soporific effects in any of the patients, as Hyadrine did, it ranked highest of all the medications in the production of central-nervous-system-stimulating reactions (27 per cent). Hyadrine, which also was responsible for nervousness (16 per cent), led all the preparations in the incidence of drowsiness (11 per cent), but on the whole this soporific effect was mild and easier to deal with than the nervous effects. The incidence of nausea was approximately the same (about 7 per cent) in patients taking Sc-7188 and aminophylline with racephedrine. Only one patient developed some nervousness and two others developed drowsiness while taking Sc-7188. Of the patients given the placebo, five described drowsiness, two reported nervousness, and one had nausea. The frequency of the placebo reactions was less than anticipated.

SUMMARY AND CONCLUSIONS

1. Two bronchodilator drugs, a new drug, and a placebo were administered to sixty-three asthmatic patients by two methods, both double-blind. In method I, the preparations were identical in appearance, identified by number, and given to each patient in sequence. In method II, they were identified by color and given to the patients together in a single bottle. The preparations were Hyadrine, aminophylline with racephedrine, and N-[β-(10-phenothiazinyl)-propyl] trimethylammonium benzene choloride (Sc-7188).

2. Hyadrine received the highest mean rating in relieving the manifestations of obstructive pulmonary disease, and aminophylline with racephedrine was a close second. The mean rating for Sc-7188 was considerably lower, and the placebo made the poorest showing.

3. When data from methods I and II were subjected to statistical analysis and compared, no significant differences in the results were found.

4. Color biases of any importance were absent in method II.

5. It is concluded that the single-bottle color-coded method of appraisal provides information equivalent to that obtained by the conventional methods.

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THE FAILURE OF ANTEPARTUM OR NEONATAL EXPOSURE TO GRASS POLLEN TO INFLUENCE THE LATER DEVELOPMENT OF GRASS SENSITIVITY

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THE factors which determine the development of specific sensitivities in atopic persons have not been determined. For example, why should one child develop sensitivity to pollen, while another in the same environment develops sensitivity to house dust or cats?

Burnet[1] has cited the work of Billingham, Brent, and Medawar[2] who observed that inoculation of mouse embryos in utero with a cellular emulsion of organs from a homozygous strain produced a specific tolerance to organ grafts of the infected strain after birth. Hanan and Oyama[3] observed that injection of bovine gamma globulin into newborn rabbits produced inhibition of antibody formation to gamma globulin in these animals after they reached maturity.

The present study was undertaken to test the hypothesis that a similar mechanism of inhibition of antibody formation by fetal or neonatal exposure of antigen might operate in human beings to affect the later development of grass pollen sensitivity. Western Oregon and Washington is an ideal area in which to test this hypothesis, since the climate is very favorable to the growth of grass and grass seeds are a main agricultural crop. Consequently, the concentration of grass pollen is very high from late May to early July, and grass pollinosis approaches the severity of ragweed pollinosis in other parts of the country. Grass pollen is also antigenically simpler than ragweed pollen. Studies by Stull, Cooke, and Chobot[4] and more recently by Augustin[5] and confirmed by this department have indicated that the major timothy pollen allergen is an albumin of molecular weight of less than 15,000 and that no active components of small molecular weight are present as they are in ragweed. Such a molecule might reasonably be expected to pass the placenta and affect the immunologic development of the fetus.

Birthdays were recorded of 150 persons who were born in western Oregon or Washington, who were under treatment for grass pollinosis, and whose symptoms began before the age of 12. The majority were children under 15 years old. These birthdays were plotted according to month and the total number of birthdays in each month was compared with the number of all births at a representative hospital of the area (Table I). Casual inspection of

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the data indicated that these birthdays were random and followed no pattern of seasonal distribution. The chi-square test showed there was no more than chance departure of the distribution of the birthdays of children with grass pollinosis from the distribution of all birthdays. If the natural exposure to grass pollen of the pregnant mother or the newborn child produced an inhibition of the later development of antibody to grass pollen, there should be a seasonal distribution of the birthdays of children who have grass pollinosis.

TABLE I. BIRTHDAYS BY MONTH OF PATIENTS WITH GRASS POLLINOSIS COMPARED TO TOTAL BIRTHS AT THE GOOD SAMARITAN HOSPITAL, CORVALLIS, OREGON 1951-1956

	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	TOTAL
Total births at GSH	325	336	385	398	384	401	318	365	414	394	356	309	4,264
Birth of pollinosis patients	16	12	17	15	13	12	16	13	14	12	10	10	150

In the plot of birthdays there should be a seasonal gap corresponding to the time in fetal development when the antibody-producing mechanism is susceptible to the establishment of a specific inhibition. No such gap was observed, and the conclusion is forced that antepartum or neonatal exposure to grass pollen does not affect the later development of sensitivity. Immunologic studies of the transfer of the pollen antigen across the placenta were planned but were abandoned when the hypothesis proved to be false.

SUMMARY

The observation that injection of antigen into fetal or newborn animals produces inhibition of specific antibody formation in the mature animal suggested the hypothesis that antepartum or neonatal exposure to pollen might confer protection against future development of pollen sensitivity. The birthdays of 150 children with grass pollinosis who were born in an area of heavy exposure to grass pollen were recorded. No seasonal distribution in their birthdays was found. It was concluded that antepartum or neonatal exposure to grass pollen is not a factor in the determination of pollinosis.

My thanks are due Drs. Merle W. Moore, Frank Perlman, and Alvin Wert who generously made data from their practices available, and to Dr. Carl Hopkins for statistical analysis.

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INSECTS AS INHALANT ALLERGENS

Consideration of Aerobiology, Biochemistry, Preparation of Material, and Clinical Observations

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"How many insects are there? And how many kinds of insects? Maybe we shall never know. But wherever we go and whether we see them or not, we are surrounded by countless millions of insects. Every forest, every field, every back yard, every roadway is a gigantic insect zoo. A wide world of endless variety and interest is open to all who will do a little investigating on their own."

—*Curtis W. Sabrosky.*[1]

INTRODUCTION

SINCE common usage has caused the word *insect* to include most of the arthropods, this terminology will be used throughout the present discussion. This will also conform with the broad title of the Insect Allergen Subcommittee of the Research Council of The American Academy of Allergy, which has encouraged many of these studies. When the class of true insects is singled out, the term *Hexapoda* will often be used.

The role of insects in allergy has been recorded, for the most part, as a series of isolated case histories in which the allergy was attributed to a variety of arthropods, chiefly of the class *Hexapoda.* The list of insects as inhalant allergens is constantly lengthening and is summarized in Table I. Reactions to biting and stinging insects are excluded from this discussion.

It would seem important, therefore, that the entire insect world be better evaluated—not only in its distribution and relationship to clinical symptoms but even further as concerns the nature of the allergenic properties. Most recently, Feinberg and colleagues[28] have aroused intense awareness of the broad nature of this problem. The Insect Allergen Subcommittee is planning to accumulate clinical experiences, biochemical analyses, immunologic studies, and entomologic data to provide a link between the obvious antigenicity of the insect allergen and clinical disorders. Coordinated efforts might eventually provide a more direct approach in understanding and treating many obscure and discouraging allergic problems.

RELATIONSHIP OF INSECTS TO ALLERGIC DISORDERS

If the postulates of Thommen[29] for pollinating plants causing hay fever are applied to insect allergens, much convincing evidence is revealed by paraphrasing these simple but basic postulates:

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1. The insect must contain an excitant of allergenic symptoms.
2. The insect must appear in sufficiently large numbers.
3. The insect must be widely and abundantly distributed.
4. The insect must be anemophilous, or wind borne, during some stage of its life.
5. The insect must be sufficiently buoyant to be carried considerable distance.

First Postulate.—Evidence that insects contain excitants of allergic symptoms is brought out by numerous specifically positive direct skin reactions and by passive transfer reactions as well (Figs. 1 and 2). There are also many convincing and confirmed reports of clinical symptoms produced by most of the major orders of the class *Hexapoda* and of clinical benefit from treatment with the specific insect allergen. The exact nature of the excitant (or excitants) is not entirely clear, and more detailed studies on the biochemical structure and immunologic activities will be elaborated upon later in this discussion.

TABLE I. ARTHROPODS INCLUDED IN OUR STUDIES

Class—Arachnida	*Class—Hexapoda (Cont'd)*
Araneae (spiders)	*Order: Coleoptera*
*Acarina[2] (mites, ticks)	*Beetles[11, 12]
	*Bean weevil[13]
Class—Crustacea	
Sowbugs	*Order: Neuroptera*
*Daphnia (water flea) (3)	Lacewing flies
Shrimp (plankton)	
Shrimp (edible)	*Order: Trichoptera*
Crab	*Caddis flies[14-17]
Class—Hexapoda (Insecta)	*Order: Lepidoptera*
Order: Orthoptera	*Moths, butterflies[18-21]
*Locusts[4, 5]	*Order: Diptera*
Grasshoppers	*Houseflies[22]
Cockroaches	*Mushroom flies[23, 24]
*Crickets[6]	*Sewer flies[25]
	*Midges[26]
Order: Isoptera	Deer flies
Termites	Black flies
Order: Dermaptera	*Order: Siphonaptera*
Earwigs	Rat fleas
Order: Ephemeroptera	*Order: Hymenoptera*
*May flies[7, 8]	*Honeybees[27]
	Bumblebees
Order: Hemiptera	Hornets
*Bedbugs[9]	Wasps
	Parasitic wasps
Order: Homoptera	Yellow jackets
*Aphids[10]	Ants

Asterisk (*) denotes those arthropods reported by others as causes of allergic rhinitis and asthma.

Second Postulate.—The appearance of insects in large numbers is evident even to the casual observer. This may be a seasonal variant not only peculiar to the climate but also predictable for certain specific insects. The caddis flies

Fig. 1.

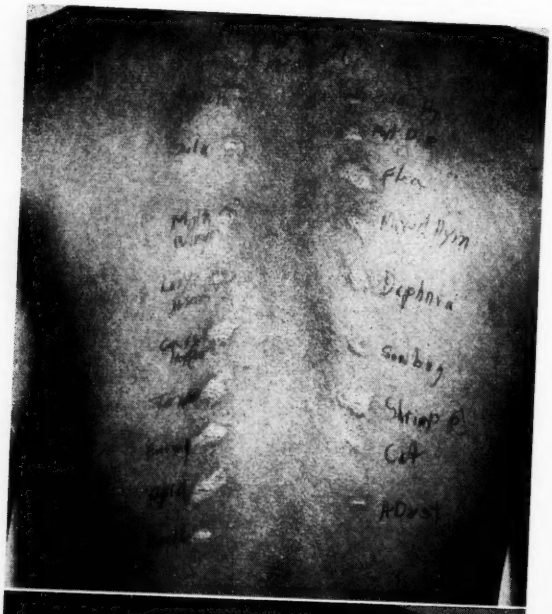


Fig. 2.

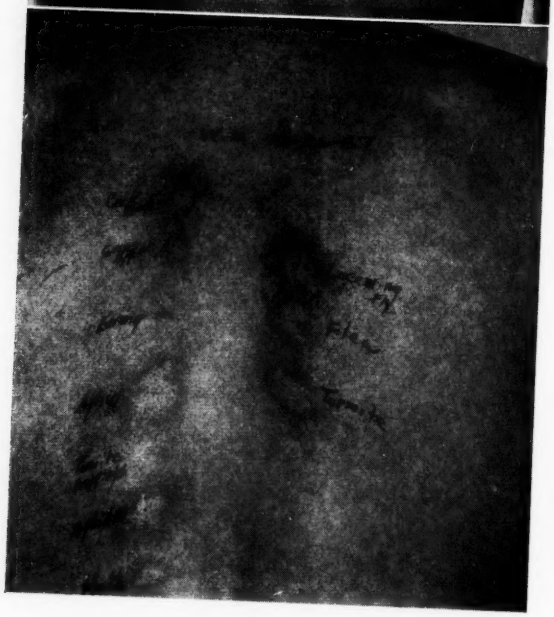


Fig. 1.—Multiple reactions by scratch method to *Hexapoda* and *Crustacea*. Note lesser reactions to cat epidermal and house dust.

Fig. 2.—Varying reactions to several hexapods. The strongest reactions are to two samples of aphids.

and May flies exemplify this very well. In temperate climates in both the late spring and the waning summer and autumn months the appearance of many such insects in what might be considered "clinical numbers" can be predicted. From Biblical times to the present age of insecticides, swarming clouds of insects have plagued us.

Third Postulate.—The wide and abundant distribution of insects has been described by explorers from the frozen arctic to the hottest equatorial regions. A volcanic island can scarcely emerge in midocean before it is inhabited by this form of life. Now, with dams and reservoirs, our own western deserts are being converted into vast breeding areas. The entire earth is filled with a terrestrial and aquatic insect fauna that lives and multiplies and dies to litter the surface and contaminate the air.

The normal population, not counting swarming or unusual increases, is at best roughly estimated for the insects living in soil. Counts in grassland in England, made to a depth of 9 to 12 inches of soil and including mites, numbered several hundred million per acre. In an oak and pine stand in North Carolina, 120 million per acre were found to a depth of 5 inches.[1]

Composition of aerial insect fauna reported by Freeman[30] showed, during a period of study, a total of 23,295 specimens represented by 421 species. Of these, 75 per cent were below 100 feet. *Diptera* were most numerous below 100 feet, while *Hymenoptera* were most numerous above this level. *Aphidae* was the dominant family at all heights. The greatest number and varieties occurred in June and September.

Of the *Hexapoda* alone, the kinds number more than a million. About two-fifths are beetles and another two-fifths include the *Lepidoptera, Hymenoptera,* and *Diptera.* The remainder comprise the final fifth. Sabrosky[1] further staggers the imagination with a few colorful observations. About 40,000 cicadas have been observed to emerge from the ground under a large tree; a queen bee may lay 2,000 eggs a day; and some termites are capable of laying an egg every two seconds, at which rate, a termite could lay a total of 43,000 eggs in a single day. A number of insects add to this prodigious multiplication by polyembryony (two or more young from a single egg). Parasitic wasps, for example, may have several thousand young emerge from a dozen eggs.

Fourth and Fifth Postulates.—These postulates are more adequately fulfilled for insects than for other aero-allergens. Not only are most insects capable of purposive (active) migration, but perhaps more important to us is the passive dispersion of these arthropods. There is scarcely an insect that at some time in its life cycle does not take to the air. Just as the ocean is adrift with marine plankton, so the air above may be filled with insect fauna that might well be named "aerial plankton."

The actual dispersal of insects is so extensive that there is less need to be familiar with those of any specific location than in the case of pollinating plants. The cotton moth of the South appears in large numbers in Canada, while the monarch butterfly, which allegedly cannot survive above subtropic temperature,

is found in large quantities as far north as the Arctic Circle and out into islands of the Pacific. Arachnids, such as spiders and gall mites, travel thousands of miles on trade winds and can be found, by air sampling aboard ships at sea, in numbers which, if applied to pollen grains, could be of clinical significance. Migrations of butterflies are especially striking. Millions of these *Lepidoptera* may fly in a swarm several hundred miles in width and traverse several thousand miles[1] over a period of days.

Felt,[31] who contributed a great deal toward a better understanding of this dispersion of insects, aptly stated: "Insects took to the airway back in the Upper Carboniferous Period, and it is not surprising that Man, a most recent traveler upon the 'wings of the wind,' should find much that is new, especially in relation to the insect inhabitants."

Dissemination of insects by air currents has been of interest to those particularly concerned with the economics of entomology. However, this may prove of significance in accounting for seasonal flare-ups of respiratory allergy. Felt,[32] Glick,[33] Coad,[34] Wolfenbarger,[35] and others described the dispersion of insects and other small organisms and made counts of the population at various levels above the earth's surface. Coad,[34] collecting insects from the level of 50 to 14,000 feet, found the densest population up to 1,000 feet. This was reduced to one-half at 2,000 feet and further reduced to one-half at 3,000 feet. After 6,000 feet, the population was lightest. Contrary to first thought, the strongest-flying insects were located closer to the ground while the smaller, weaker ones were found at higher altitudes. Wingless insects were collected at high altitudes of more than 10,000 feet. It is evident from this that travel and dispersion were partly voluntary and to a great extent involuntary through the rising air currents.

Glick[33] and Felt[32] enumerated meteorologic conditions which might affect and thus even predict the concentration of insects:

1. *Temperature.* This is most important. The optimum is 75° to 80° F. There is a rapid drop in count with temperatures over 90° or under 70° F.

2. *Humidity.* Numbers increase as absolute humidity rises. Optimum dew point is about 62° F.

3. *Barometric pressure.* This has a varying effect. Some insects become nervously active at low pressures (approaching storms); others swarm at higher barometric pressures. Crickets and other insects are often found in vast numbers on the western shores of Lake Michigan after a storm.

4. *Wind velocity.* This is also a complex subject. Insects, especially moths, migrate with the prevailing winds that are favorable. Winds of high velocities at lower levels are of minor importance. Convection currents and other upward air streams carry large numbers of insects (especially those weak of flight) to high altitudes of 15,000 feet and more, where horizontal wind velocities may be 100 to 200 miles an hour, carrying this "aerial plankton" many hundreds and even thousands of miles.

The physiographic aspect of a region affects the total insect count. Streams, lakes, oceans, and mountains[36] contribute much to this. The winds follow valleys and rivers. A change in temperature of the insect-laden winds may cause large numbers to appear on the edge of a lake in a mountain resort where hay fever sufferers have sought refuge. Temperature and moisture affect not only the growth of insects but also the vegetation upon which they feed. Thus, in his deforestation and extensive irrigation projects, man has indeed altered the flora and at the same time the insect fauna.

The invasions of homes by insects are frequent experiences that may call for thorough fumigation by professional exterminators. The occurrence of asthma as a result of such invasions has been reported.[9, 21, 22] The more casual occurrence of insects in homes and places of work may presumably be even more prevalent. Felt and Chamberlain[37] studied this problem in tall office buildings. Every[38] reports the steady invasion of homes by many insects seeking warmth from approaching inclement weather. Among these invaders he includes strawberry root weevils, grass weevils, box-elder bugs, grass bugs, and leaf hoppers.

This discussion of dissemination and concentration of insects has not emphasized the insect debris that must accumulate from successive generations that have bred and died and become a part of our dust in a single season. In sheltered areas, as well as in homes, this may become an accumulative factor of several seasons.

The great obstacle in measuring this aerial fauna and the insects within homes is the lack of a method for readily identifying such insect debris by air sampling in a manner comparable to that used for pollens. Slide counts of insect hair, scales, and other debris[39] have been of little clinical value. Identification of air or dust samples by other physical means has been disappointing. Detection of the insect cuticle by fluorescence and phosphorescence cannot be considered distinctive since other compounds, including albumins, gelatin, and keratin, are similarly fluorescent.[40] Spectroscopic examination of dust samples containing insects has likewise given little promise. Thus far, only a few studies have been done with the absorption or transmission of infrared irradiation. When some technique is devised for even a gross quantitative estimate of insect material in the air and dust, then we may feel that we are moving forward to the better evaluation of insects as inhalant allergens.

NATURE OF INSECT ALLERGENS

General Consideration.—Little is known of the nature and number of excitants that produce immediate wheeling and are capable of producing antibodies that may readily be passively transferred. There is reason to believe that two or more such excitants exist. It is important to know whether these are common to the entire Phylum *Arthropoda* or are specific for the class, for the order, for the family, or even for the species. Gaillard,[10] working with aphids, felt that the protein in the exoskeleton varied with insect species and, since he presumed that the protein was the antigenic element, he concluded that insect allergens were species specific. This is, indeed, a frightening presumption

and it is hoped that a common denominator can be found to permit grouping of such allergens by families or, even more broadly, by order within each class in the Phylum *Arthropoda.*

Chemistry.—The integument of the *Arthropods,* like the epidermal tissue of birds and mammals, is generally presumed to contain the allergenic substances. In preparing insect allergen, however, the entire organism usually has been used—thus throwing some doubt on the propriety of applying our knowledge of the chemical nature of the insect cuticle to the allergenic extract used in most clinical studies, even though the wings and exoskeleton of most insects make up the great proportion of the dry weight of these organisms. Nevertheless, some light may be shed by reviewing what is known of the chemistry of this exoskeleton.

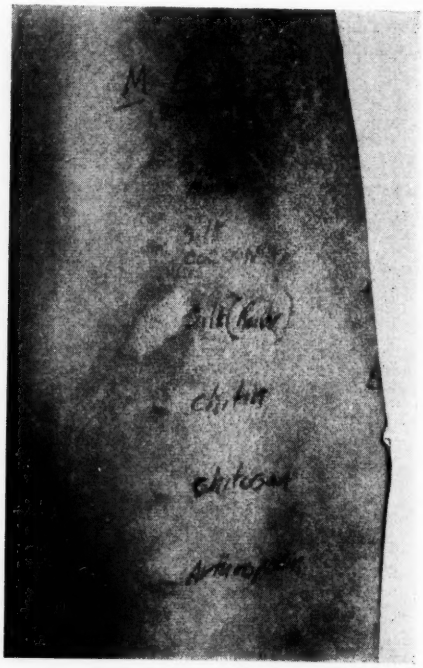


Fig. 3.—Strong reaction to silk. Moderate reaction to silk moth and silk cocoon. Negative reaction to chitin and arthropodin.

The insect cuticle possesses protein, carbohydrates, and sulfur-containing materials.[41, 42] The proteins, which are of most interest, are generally large and chemically possess similar properties for many of the insects studied. These proteins change in the course of development from the larval to the adult stages. For example, their solubility is greatly reduced during pupation. Similar chemical properties, however, cannot be literally translated as allergenic similarity. The substances chitin, arthropodin, and sclerotin have been identified in varying proportions in the cuticle of most insects.

Chitin: This substance has been implicated frequently in insect allergens;[10] yet, this is only one component comprising from one-fourth to one-half of the dry weight of the entire cuticle. Although some investigators[43-45] have studied the protein in chitin and it has been considered to exist in minute amount as a polysaccharide-protein complex, there is much evidence that would cast doubt on the probability that chitin is implicated in the allergenicity of insect extracts used in clinical testing (Figs. 3 and 6). It is quite probable that allergists are erroneously using the term *chitin* in place of the term *cuticle.* Chitin is quite insoluble, resembling cellulose in this respect (and in others). Chitin, for example, differs from silk in that the latter is secreted by glands derived from the epidermal layer, while chitin itself, although crystallized in a similar way, is secreted by glands of hypodermal origin, resembling synovial fluid of the vertebrates. This may explain why both chitin and synovial fluid are capable of calcification. It must be presumed, however, that although chitin is extremely insoluble, some changes must occur in nature. The minute copepod crustacea produce several billion tons of chitin annually,[46] while other marine crustacea and the terrestrial arthropods produce several times this astronomic amount. It is evident that if this insoluble substance and other components of the cuticle were not decomposed, soon the earth's available elements, especially carbon and nitrogen, would be seriously depleted. Evidence of chitinoclastic activity by a variety of bacteria and fungi through enzymatic action has been demonstrated by a number of authors.[47, 48] Whether or not an intermediate product in this breakdown is allergenic remains a moot question.

Chitosan.—This is one of many derivatives of chitin and is produced by fusion with alkali at high temperatures. It does not occur naturally. It has low nitrogen contents (Kjeldahl). Results of clinical testing with chitin and chitosan will be discussed subsequently.

Arthropodin.—The proteins of the insect cuticles which are presumably the allergenic substances have been inclusively called by the ill-defined term *arthropodin.* Many investigators recognize several protein fractions. Fraenkel and Rudall[45] first proposed to this water-soluble fraction the name *arthropodin* "on account of its universal occurrence among arthropods' limbs." Hackmann[49] and his associates, in recent work, have shown that arthropodin is heterogeneous and that arthropodins from different insects give different electrophoretic patterns. They believe that there may be some protein fraction or fractions common to all insects. Their proteins extracted by the borate buffer and those extracted by water are not (as formerly thought) identical. They found that water-soluble protein may account for as little as 15 per cent or as much as 50 per cent of the protein in the insect cuticle. The arthropodin made available for our studies was a protein extract from larval cuticle of *Diaphania dorsalis Don.,* prepared by a borate buffer of pH 9.

This group of proteins has certain characteristics:

1. It is quite soluble in water.
2. It does not coagulate in hot water.

3. It is precipitated by ethyl chloride above 45 per cent and by one-half saturated ammonium sulfate and saturated sodium chloride as well as cold trichloroacetic acid.

4. After precipitation by trichloroacetic acid and alcohol, it is readily redissolved.

5. Boiling produces molecular configuration of denaturation, but the protein remains soluble in water.[50]

6. Partial analysis of arthropodin shows high tyrosine and low glycine but no amino acids containing sulfur. In this way it resembles sericin.[40]

Sclerotin.—The commonly occurring water-*in*soluble protein was termed *sclerotin.*[44] This substance can be removed by 5 per cent potassium hydroxide solution. The soft cuticle contains a high percentage of water-soluble fraction; in the hard cuticle, however, the larger percentage is represented by this water-insoluble protein. Sclerotin is still not well understood, although it is thought by some to be a derivative of arthropodin in the development of the insect. It contains less nitrogen but, on the other hand, it does contain sulfur and carbohydrate. To the allergist, this is an important point. If the proteins of the larval or pupal stages are allergenically identical to that of the adult stage (but a more water-soluble protein is available), then it would be advantageous to prepare the allergen from the preadult stage of the insect to be studied. Benson,[56] however, in studying hypersensitivity to the mosquito, found that although the newly hatched species reacted in a manner similar to the reaction obtained with the older insects, the pupae failed to incite skin reactions in a sensitive patient. Further studies on other insects in various stages of development are in progress.

What part these proteins play in the total allergen is not entirely clear. In the case of arthropodin, several insects *(Crustacea* and *Arachnida)* contain this water-soluble fraction with the same general characteristics[44] and have similar chemical and electrophoretic patterns.[49] Such a finding might stimulate a further search for the common allergens.

Silk.—This cannot be included, in the strict sense, in the insect cuticle, although it is produced by cells of ectodermal origin homologous with the simple epidermal cells that produce various surface structures, including hair and cuticle. Silk is produced by many insects, including spiders, *Lepidoptera* (larval stage), and most other orders within the class *Hexapoda.* These include *Trichoptera, Siphonoptera, Hymenoptera,* some *Coleoptera, Diptera, Homoptera, Corrodentia, Neuroptera,* and *Embiidina.*[40] This number may still be incomplete, but the listing of so many common insects capable of producing a silk may well account for the frequency with which some patients react to almost all of the insect allergens used in testing (Fig. 1). Silk may be, after all, but one allergenic link between insects.

The raw silk produced by the larva of the silk worm moth, *Bombyx mori,* consists of about 75 per cent fibroin and 25 per cent sericin (silk gelatin). The sericin which acts as a cement for the two strands of fibroin to produce the

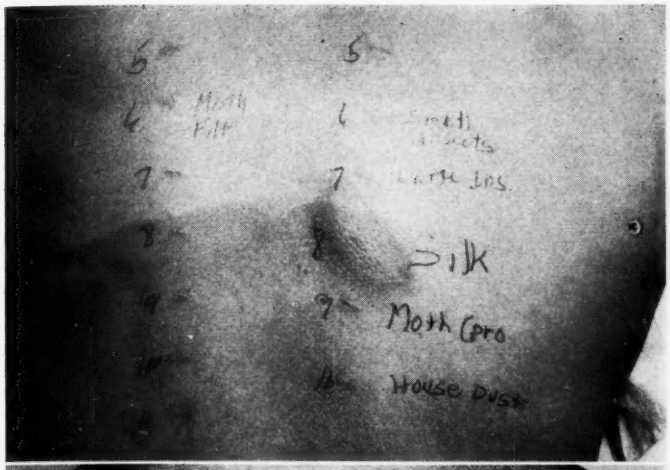


Fig. 4.

Fig. 5.

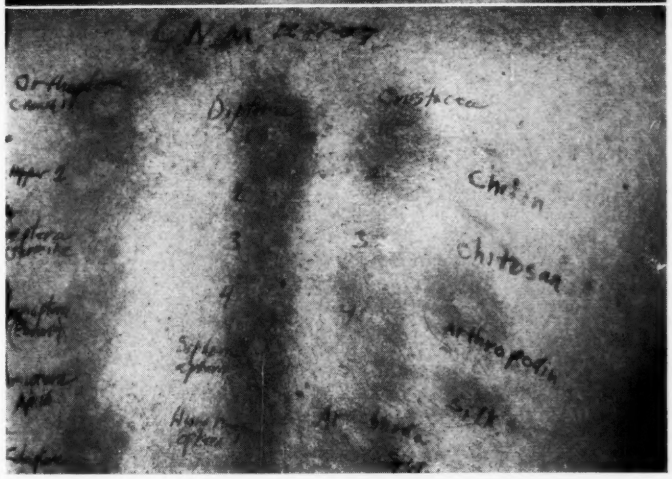


Fig. 6.

Fig. 4.—Large reaction to silk. Negative reactions to all insects, house dust, and epidermals.

Fig. 5.—Strong reaction to moth "protein." Moderate reaction to moth filtrate. Negative reaction to silk. Moderate reaction to moth-wing solution prepared eleven years previously (October, 1946). Negative reaction to moth extract from commercial laboratory (solution made in October, 1957).

Fig. 6.—Strong reactions to arthropodin and to insects. Only slight reaction to silk and negative reaction to chitin.

silk filament is water soluble and removed in the course of processing the fiber. With its lack of commercial value, it has received little study as to its chemical nature. Recently, however, it has been found to contain two separate albumins with different physical and chemical properties.[51] Such findings certainly do not help to simplify the search for a common allergen in the silk of the various insects. Recent reports by Coleman[52] and by Friedman and associates[53] of severe allergic reaction to a vaccine filtered through silk add emphasis to its allergenic properties. Presumably, the water-soluble sericin was not entirely removed or some other allergenic substance was responsible. (Their inability to exhaust the sensitized site with silkworm extract, however, permits too many possibilities to offer any rational interpretation.)

It is well to point out again the hazard involved in applying chemical studies to allergenicity as demonstrated by skin testing on sensitive patients or passively sensitized skin sites. The skin test demonstrates differences in the various water-soluble and water-insoluble fractions from the whole insect and from insect cuticle. Figs. 4, 5, and 6 show reactions to silk only, to the whole insect only, and to arthropodin and not to silk, among many other variables in the course of patient testing. Chitin and chitosan failed to react on all occasions.

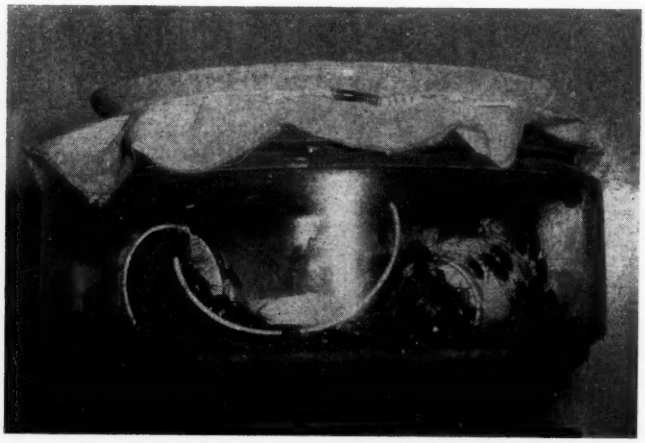


Fig. 7.—Breeding jar for propagation of cockroaches in large numbers.

SOURCES OF INSECTS

The gathering of insects required many hands to provide representatives of the various classes, orders, families, and even species within the Phylum *Arthropoda,* and in sufficient numbers to permit the preparation of an adequate supply of extract. Some were grown in the laboratory. These include cockroaches (Fig. 7), mosquitoes, flies, ticks, parasitic wasps, and fleas. If the need should warrant, perhaps many others could be thus provided. Other insects were gathered in large numbers on a single field trip. This was particularly true of the swarming varieties. Those insects that live solitary lives required

more patience and many additional hands. The neighborhood children proved enthusiastic assistants, who, in the course of an afternoon field trip, gathered large numbers of insects which were later segregated.

Insects within the Class *Crustacea* were acquired in various ways and included Daphnia, minute brine shrimp (plankton), and sow bug or pill bug *(Armadillidium vulgare)*. The *Daphnia* were readily gathered with a dip net from a large stagnant casting pool. The catch was especially abundant in late spring and early autumn. The brine shrimp were raised in myriads from eggs purchased in a tropical fish store. However, this plankton can be obtained freshly frozen from this same source. Sow bugs are abundantly present in damp areas under rocks and logs and in places where wood touches moist dirt. On a single Sunday afternoon, even in early spring, many thousands were quickly gathered, leaving the impression that their bodies and skeletons must certainly contaminate the uncultivated earth and the unexcavated cool, damp areas beneath homes.

A practical and inexpensive apparatus for gathering large quantities of insects is the light trap. In a short period of several nights, a pint of insects was caught (Figs. 8 and 9). The process of separating them according to orders was tedious but less time-consuming or costly than attempting to collect so many in the field. The greater share of this material obtained in the light trap was processed without segregation, labeled "mixed insects," and provided excellent material for screening patients for later detailed testing and for specific treatment. A highly allergenic and concentrated "protein precipitate" was made from one such "mixture" of insects.

In the past, handling insects before preparing the final extract was simply a matter of storing under desiccation the various specimens gathered. Osgood[16] describes collecting his caddis flies in a live state during flight, as well as sweeping up large quantities that had dried in the summer air. Evidently both provided excellent sources of allergenic extract. This insect debris, after several months in a dried state, was still capable of inducing asthmatic symptoms in the sensitive patient who chanced to inhale it. Gathering and storing in the simple dried state such insects as caddis fly, May fly, housefly, mosquito, and moth may be satisfactory. However, any slight amount of moisture will quickly result in mildew and make worthless the entire contents. In storing the whole insect, bacterial action may continue, resulting in some putrefactive changes so that most insects stored as they are gathered, even under desiccation, soon develop the characteristic unpleasant odor. This is particularly true of the large-bodied insects, such as the beetle, grasshopper, bee, hornet, and yellow jacket.

To avoid this spoilage, the method was followed of processing from the live stage or quick freezing and storing insects under subfreezing temperature.[54] The latter method of storage was necessary for those specimens that could be gathered only a few at a time (spiders, solitary wasps, etc.). Insects processed from the live or quick-frozen stages yielded a final product that was essentially odorless.

Fig. 8.

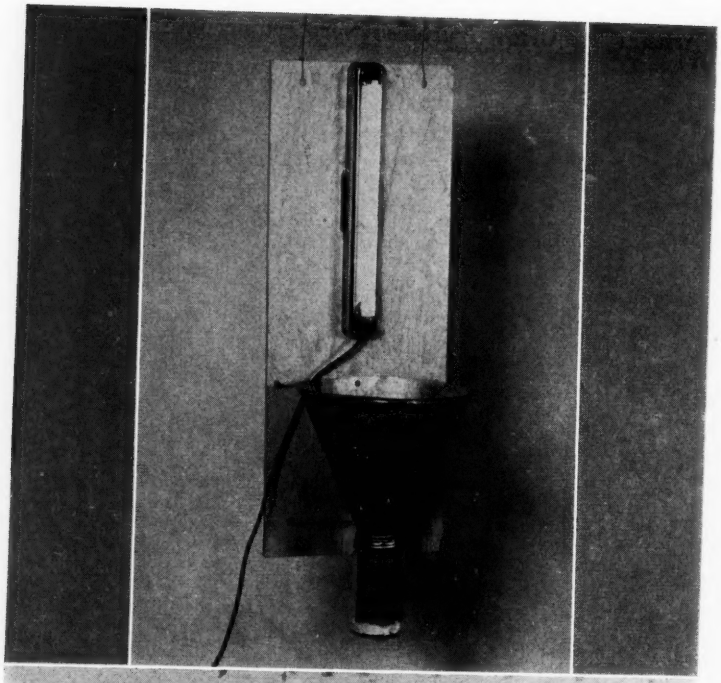


Fig. 9.

Fig. 8.—Light trap, fluorescent tube, and funnel through which insects drop into screw-top jar with inner wall screen. At bottom of jar is gauze soaked with carbon tetrachloride.

Fig. 9.—Three-day "catch" in light trap (almost one pint by volume). Most of the common important hexapods are represented.

PREPARATION OF INSECT ALLERGENS

The procedure in preparation of these allergens must necessarily vary with each type of insect used. Among the many reports in the literature of testing and treating with insect allergens, usually perfunctory comments are offered as to their preparation. In most cases it must be presumed that a simple concentrated filtrate was used. Gaillard[10] defatted aphids with toluene and extracted with Coca's solution. This extract was further dialyzed, filtered, and standardized according to its total nitrogen content. He reports 0.16 mg. total nitrogen per milliliter (T.N./ml.), which is rather low in comparison with analyses of our extracts. The dark brown color he describes, if gradual in development, may have been the result of the action of phenol in Coca's solution and must be reckoned with in the stability of this aqueous allergen. Osgood,[17] working with the Caddis fly, also defatted and finally standardized the aqueous filtrate on the basis of protein nitrogen. Frankland,[5] working with locusts, defatted with acetone and extracted with buffered saline. He found the feces of locusts a potent allergen. Benson and Semenov,[55] working with the honeybee, prepared separately from the body and from the stinging mechanism a "protein" precipitated out by saturating the aqueous extract with magnesium sulfate. Dialysis for thirty-six hours gave two fractions—soluble and insoluble, respectively—which they presumed to be the albuminous and the globulin fractions.

It is evident that, despite the diverse techniques, similar fundamental principles are employed: first defatting and then extracting with isotonic buffered aqueous solution. The final extract obtained is usually concentrated. Such extracts, in addition to being water soluble, are insoluble in alcohol, nondialyzable, and thermostabile,[56] and contain no significant nonspecific irritants. The protein-nitrogen content is appreciable and is often used as a guide in standardization. No studies have been done on the stability of these extracts. Since the entire body of the insect is used, it is possible that certain enzymes may be carried through during the extracting process, thus later slowly altering the active protein to a split nonprotein product which has less allergenicity. Therefore, perhaps a dried or lyophilized state would offer material that would be or remain stabile and could be stored for an indefinite period of time.

The method of preparing insect allergen material used in our study has been repeatedly modified over a period of several years, but it has recently been standardized. All insects processed from a live or quick-frozen state are defatted with ethyl ether—some with low fat contents *(Orthoptera)* by simple overnight soaking and others with very high fat contents *(Hymenoptera)* by Soxhlet's apparatus. The insect bodies must best be broken up to obtain thorough defatting. Without adequate removal of the fatty substances, filtering of the final aqueous extract proved tedious and discouraging. After defatting, this is further ground by hand with mortar and pestle and placed in a flask to be lyophilized for twenty-four hours. The material is then finely ground in a

Wiley mill, using a 60- to 80-mesh screen, following which the fine powder is placed in the lyophilizer for another twenty-four hours. The final product is stored in a desiccator indefinitely without fear of mildewing or deteriorating.

Fig. 10.

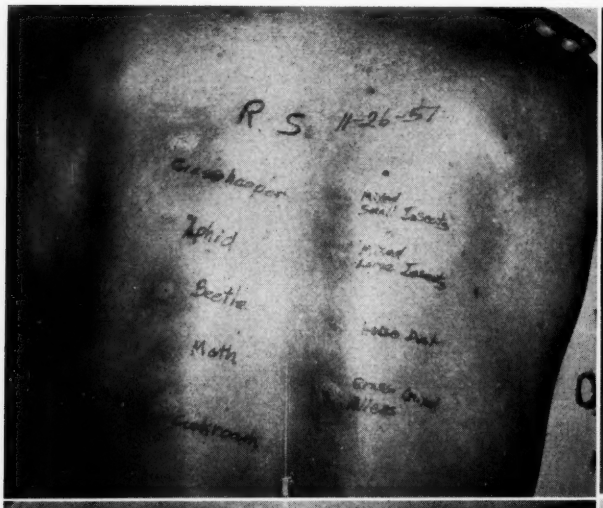
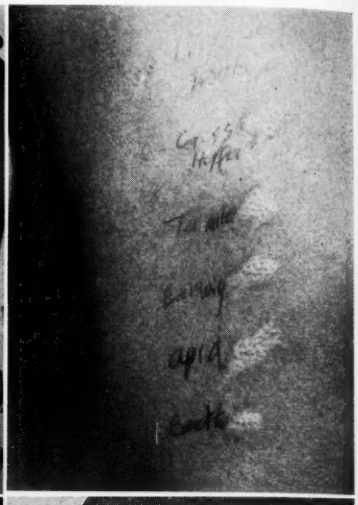


Fig. 11.



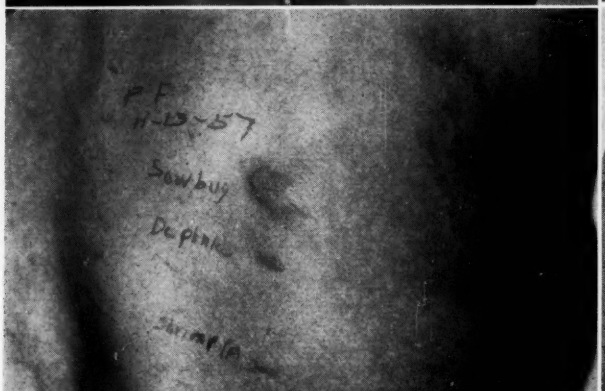


Fig. 12.

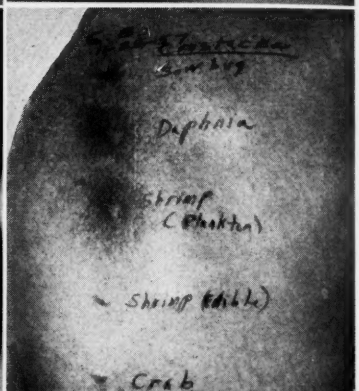


Fig. 13.

Fig. 10.—Selective reactions to insects and to pollens. Negative reactions to aphid, moth, and house dust.

Fig. 11.—Selective reaction to several *Crustacea*. Strongly positive to sow bug and crab. Slight reactions to shrimp and *Daphnia*. (The reverse is seen in Fig. 12.)

Fig. 12.—Moderate positive reaction to minute fresh water and salt water *Crustacea*. Negative reactions to other *Crustacea* (the reverse of Fig. 11).

Fig. 13.—Positive scratch reactions to termite and earwig, among others.

A few insects were gathered in sufficiently large numbers to permit the preparation of a "protein powder." This was made by adding to the crude aqueous filtrate four parts "ice cold" acetone. The precipitate was dried and pulverized and made ready for redissolving in small amounts as needed. This

procedure was followed in preparing the "protein precipitate" of moth, mosquito, bee, and a "mixture" of small insects recovered from the light trap. As was expected, powdered precipitate in the case of each insect had a higher protein-nitrogen content and a stronger whealing effect than simple aqueous extract (Fig. 5). It is an ideal procedure of course, for preparing good insect allergen but is practical only when an adequately large quantity of original material can be gathered.

For later preparation of an aqueous extract, a small weighed amount of lyophilized fat-free material is dissolved in ten parts buffered 50 per cent glycerin by weight to volume and extracted for forty-eight hours at room temperature, shaking several times a day. (Some insects require greater dilution.) This is then centrifuged slowly and the supernatant solution is passed through a sterilizing Seitz filter. A portion of the final filtrate is used for scratch testing. The remainder is stored in sterile bottles for further dilution to be used in intradermal testing and treatment. This glycerinated buffered extract has, for some insects, retained its allergenicity now for many years. For example, with such extracts of moth wings in buffered glycerin, a strong whealing reaction was obtained by the scratch method after eleven years (Fig. 5).

The method thus outlined in some detail for preparation of concentrated filtrate is not at all laborious. It is superseded in quality only by precipitating out the "protein" with cold acetone. However, the latter procedure for the present is practical only with a few insects. If, in the future, it is found that some allergen or allergens common to all or many insects exist, then a precipitation method of preparing a "purified protein" can be used on insects that can be gathered in large quantities to yield such basic materials. As evidenced in these studies, arthropodin, silk, and chitin still do not account for all the allergenic substance in the insect; nor can we depend on tests with one order to be interpreted as tests of sensitivity for another (Figs. 10, 11, 12, and 13).

BIOCHEMICAL ANALYSES OF INSECT ALLERGENS

Total nitrogen and protein-nitrogen studies were not done routinely, but Table II shows determinations on several unselected extracts prepared as filtrates and as "protein precipitates." The samples used for analysis were in 50 per cent buffered glycerin which had been passed through a sterilizing Seitz filter and was ready for skin testing. Each of these samples was submitted to three analyses. Phosphotungstic acid was used to prepare them for nonprotein-nitrogen determination. After Kjeldahl digestion, the flask contents were made alkaline and the ammonia steam was distilled into boric acid which was then titrated further against 1:100 normal hydrochloric acid. There was no more than 6 per cent variation in these three analyses, and the average was recorded in Table II. The "protein precipitate" in a dilution of 1:25 by weight volume, as would be expected, showed higher total and protein nitrogen contents. The large-bodied insects, such as the cockroach and grasshopper, as simple filtrates in a 1:10 dilution approximated the more concentrated extracts in the protein-nitrogen contents. The nonprotein-nitrogen contents in all samples accounted for more than one-half the total nitrogen determination of this water-soluble

material. Quite probably that does not represent alterations of the protein. We are dealing here with a crude aqueous extract of the whole insect, and a better understanding of this nonprotein nitrogenous material must await our further studies.

TABLE II. NITROGEN CONTENTS (PROTEIN AND NONPROTEIN) OF CERTAIN INSECT ALLERGENS AND SILK

ALLERGEN	DATE SOLUTION MADE	DATE OF ANALYSIS	TOTAL NITROGEN (MG./ML.)	NON-PROTEIN NITROGEN (MG./ML.)	PROTEIN NITROGEN (MG./ML.)
Silk (raw) "protein" 1/25	11-23-57	12-17-57	1.500	0.215	1.285
Small insects (mixed) "protein" 1/25	12-16-57	12-17-57	4.497	2.820	1.677
Small insects (mixed) filtrate 1/10	4-10-57	12-17-57	3.070	2.285	0.785
Moth "protein" 1/25 ("old")	10- 8-56	12-19-57	1.643	1.000	0.643
Moth "protein" 1/25 ("fresh")	12-16-57	12-19-57	2.285	1.248	1.037
Moth filtrate 1/10	12-16-57	12-19-57	2.070	1.535	0.535
Moth filtrate 1/10 (commercial lab.)	10-25-57	12-19-57	0.7150	0.5700	0.1450
Cockroach filtrate 1/10	12-18-57	12-27-57	3.177	2.213	0.964
Grasshopper filtrate 1/10	12-18-57	12-27-57	3.177	1.713	1.464

Analyses of the four samples of moth extracts revealed several interesting points:

1. The "protein precipitate" contained more protein nitrogen and less nonprotein nitrogen than the simple filtrates.

2. "Protein" prepared on Oct. 8, 1956, and that prepared one year later on Dec. 19, 1957, were from the same dried powdered material but were prepared separately in solution form; thus, a positive statement of reduction in protein nitrogen cannot be made.

3. It is interesting to note that of the two "protein" samples there was no striking increase in nonprotein nitrogen after fourteen months, which may be some index of stability of this material in buffered glycerinated solution.[58]

4. The moth filtrate from a commercial laboratory was much lower in protein nitrogen and relatively high in nonprotein nitrogen. This is reflected in the lack of whealing on known sensitive patients (Fig. 5).

It must be reiterated that these analyses can offer no fundamental conclusions but are early and interesting observations made in the course of our studies.

CLINICAL STUDIES

Abundant clinical and immunologic evidence has shown that a number of insects act as inhalants to produce symptoms of hay fever and asthma (Table I). An attempt at clinical correlation of such symptoms with the rest of the

insect fauna, with its emanation and with insect debris, becomes a more difficult task. Many conclusions must be drawn by inference, while the final acceptance will have to await the results of vast clinical experiences over a period of years.

As patients are studied more thoroughly with the thought that most insects may be inhalant factors, much provocative evidence is revealed. This clinical problem has been passed over lightly—not only because of failure to use the insect allergens more routinely in testing of patients but, more important, because the few such allergens available commercially have often proved virtually inert. This was confirmed recently after testing known sensitive patients with some of these allergens of questionable worth (Fig. 5).

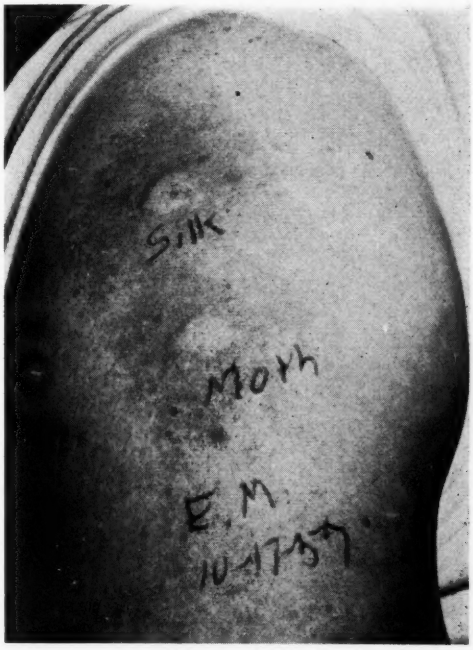


Fig. 14.—Typical reactions to both silk and moth extracts.

At the onset of these studies, testing was performed only on those patients with a history of seasonal symptoms but with negative reactions to pollens. In none of these twelve or more patients were there significantly positive reactions to insect allergens. Next, it was found that patients sensitive to silk often reacted to such insects as moths (Fig. 14), while those sensitive to crab and lobster, reacted to *Daphnia* and to the minute ocean plankton (shrimp). In these patients, when more insect allergens were tried, frequently many positive reactions resulted (Fig. 15).

The major portion of our clinical studies, however, is based on routine testing with insect allergens on consecutive unselected patients who were referred because of a history of allergy or presumptive evidence of an allergic disorder. The number and variety of insect allergens applied increased in the course of

the studies to encompass almost all of the representative arthropods, including the classes *Crustacea, Arachnida,* and *Hexapoda.* In the last class, especially, the important orders were represented by one and sometimes by several families and species.

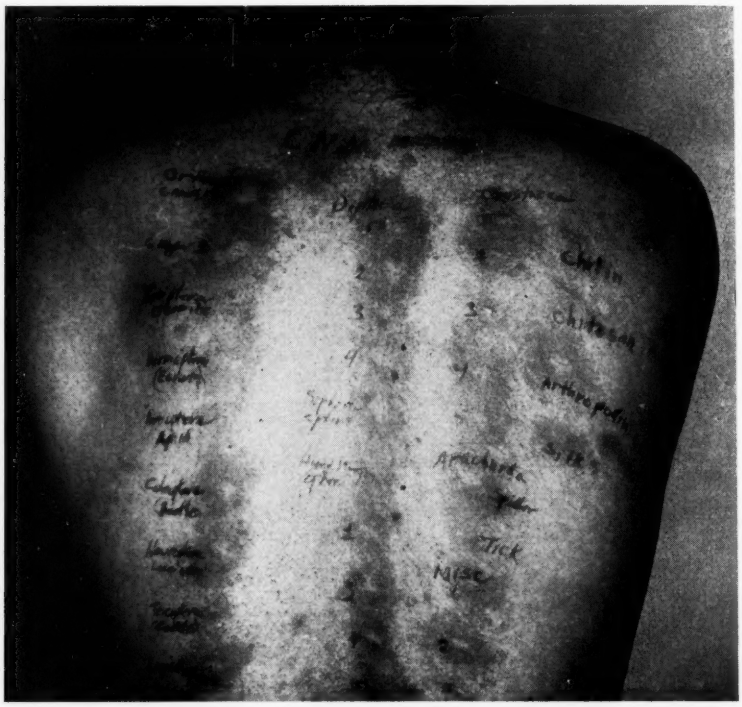


Fig. 15.—Strong reactions to most of the orders within the class *Hexapoda* to *Crustacea, Arachnida,* and *Arthropodin.* Note slight reaction to silk and negative reaction to chitin.

Correlation was attempted with some of the more basic substances of insect origin, including chitin, chitosan, arthropodin, sericin, and fibroin. Raw silk was used as a presumable source of sericin and possibly of fibroin. A few milligrams of arthropodin were obtained from moth larva *(Diaphania dorsalis Don.)* and supplied by Dr. R. H. Hackman of Canberra, Australia. Since in the course of these studies new insect allergens were added and new methods of preparing material were developed, it would be difficult and almost valueless to offer an absolute statistical summary of results. At the start, only the scratch test was employed. Later, selected patients were tested directly by the intradermal method. Because of several systemic reactions, however, a routine of scratch testing first was established. The intradermal test was used for confirmation of slight or equivocal reactions resulting from simple skin scarification.

A total of 457 consecutive, unselected patients were tested with available material. With the first 205 patients, inadequate material and incomplete information limited the use of such data to a series of impressions that led to more formalized procedures of preparing allergens, testing patients, and recording

results. The first 205 cases, therefore, are not included in the tabulation to follow. The remaining 252 consecutive patients studied provided more concrete conclusions, and eighty-three of those patients are now receiving injections of insect allergens. At the conclusion of these studies, the records of the last 252 patients were tabulated for the following:

1. Age and sex incidence.
2. Geographic distribution, occupation, and race.
3. Relationship to clinical diagnoses.
4. Relationship of skin reaction to insects and to other inhalant allergens.
5. Interrelationship of insect allergen reactions.

In general, it was found that approximately one-half of the patients tested routinely by the scratch method reacted to several of the insect allergens (Table III). When intradermal testing was done to confirm equivocal scratch tests, the percentage of positive reactions rose above 65 per cent. In that group on whom only intradermal testing was done, the percentage of positive reactions was understandably low. These patients were tested directly by the intradermal method because they were presumed not to be reactive to any allergens. Most of them were in the older age groups. In some instances we were surprised by very large local and several systemic reactions.

TABLE III. INCIDENCE OF SKIN REACTIONS TO INSECT ALLERGENS
(ANALYSIS OF 252 CONSECUTIVE UNSELECTED PATIENTS)

METHOD OF TESTING	TOTAL TESTED	NEGATIVE TO ±	+ ++	+++ ++++	TOTAL POSITIVE	PER CENT POSITIVE REACTION
Scratch only	41	20	3	18	21 + − ++++	50
Scratch and intradermal	86	56	11	19	30 + − ++++	35
		19	10	57	57 +++ − ++++	66
Intradermal only	125	51	30	39	39 +++ − ++++	31

Sex, in relation to insect allergen, might have reflected more the difference in occupation and exposure of patients to insect debris. However, there was a remarkably even division between the sexes as to those who did and those who did not show reactions (Table IV). This again may point to the ubiquitous nature of the insect allergen.

Age variation is shown roughly in Table V. Although small children were often tested by the scratch method only, and those in older age groups by intradermal methods directly, some gross observations can be made. It is evident

TABLE IV. INCIDENCE OF POSITIVE REACTIONS TO INSECT ALLERGENS IN RELATION TO SEX

SEX	TOTAL	NEGATIVE	POSITIVE
Males	146	74	72
Females	106	53	53

that, although about 50 per cent of the entire group showed positive reactions, those in the early age groups and those over 45 years of age fell much below this, while those between the ages of 16 and 35 showed a frequency of reactions to insects well above the 50 per cent average.

TABLE V. INCIDENCE OF POSITIVE REACTIONS TO INSECT ALLERGENS IN RELATION TO AGE

AGE (YEARS)	TOTAL TESTED	SCRATCH ONLY		SCRATCH AND INTRADERMAL		INTRADERMAL ONLY		TOTAL	
		NEG.	POS.	NEG.	POS.	NEG.	POS.	NEG.	POS.
5 and Under	14	8	–	–	4	–	2	8	6
6-15	47	7	5	10	12	4	9	21	26
16-25	27	–	3	3	11	2	8	5	22
26-35	44	1	6	5	12	9	11	15	29
36-45	53	5	3	2	11	22	10	29	24
Over 46	67	3	1	7	11	28	17	38	29
Total	252	24	18	27	61	65	57	116	136

GEOGRAPHIC DISTRIBUTION OF PATIENTS SENSITIVE TO INSECT ALLERGENS

The patients studied were distributed over a vast area of the Pacific Northwest, including the states of Oregon, Washington, and Idaho. There were also a number of patients from northern California, Nevada, and western Montana. All varieties of climate, altitudes (from sea level to 8,000 feet), occupations, and population densities were represented. The population in the Pacific Northwest has increased in the past ten years by more than 40 per cent, representing migration from all corners of the United States, Central America, Europe, Africa, and the Orient. Patients from London, England, to Siam, and from Ghana to Melbourne, Australia, are represented and showed reactions to insect allergens.

In these studies, all patients from the above-enumerated areas of the Pacific Northwest were proportionately represented without any striking predominance of any localities or occupations. In spite of these diverse meteorologic conditions and racial groups, no one group revealed any higher percentage of patient reactions to insect allergens than another. This is certainly in contrast with pollen sensitivity, which conforms with the flora of the region in which the patient is living or has lived for a number of years. Perhaps a lack of conformity of insect-sensitive patients to race and region is further evidence of the universal distribution of orders of the *Hexapoda,* as well as other classes of arthropods.

It has, been reported of course, that in areas in which insects appear abruptly and in large numbers, these insects prove to be responsible for acute symptoms. This is best exemplified in the Great Lakes areas (caddis fly and May fly). Such proved seasonal problems should stimulate more intense investigation in other areas that may well produce such allergenic conditions in less obvious proportions. The cicadas *(Homoptera)* in the Midwest, the lady

beetle *(Coleoptera)* in the Southwest, the inevitable moth and butterfly *(Lepidoptera)*, and the cricket, grasshopper, and locust *(Orthoptera)* in other areas which are periodically plagued will merit closer scrutiny.

Another area for investigation might well include those regions where the minute brine shrimp is abundant. This would designate the San Francisco Bay

Fig. 16.

Fig. 17.

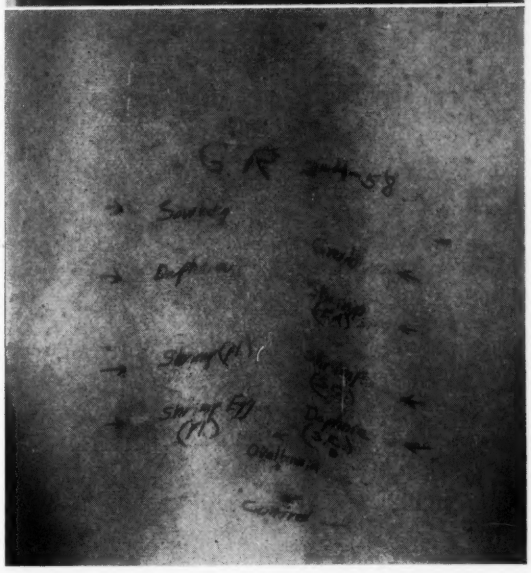


Fig. 16.—Strong reactions to all *Crustacea* extracts tested. Negative reaction to shrimp (plankton) eggs.

Fig. 17.—Strong reaction to shrimp (plankton) eggs. Negative reaction to all *Crustacea* extracts and ovalbumin.

area, the Gulf of Mexico, the Salt Lake basin, and other salt water areas where myriads of these minute organisms fill the shallower waters. In addition, their eggs, almost dustlike in size and color, which are gathered and packaged for hatching to be used as food for tropical fish, may also be inhalant factors in these areas.

Crustaceans are common ingestant allergens and violent reactions are not infrequently observed. In our studies, however, the *Crustacea* and its subclasses and orders as insect allergens were also explored. This included *Daphnia* of fresh water, minute salt water shrimp, and terrestrial crustaceans (sow bug). In general, positive reactions to this class occurred less frequently than to other arthropods, but they did occur frequently and violently in patients who were otherwise sensitive to crustacean food. In a number of circumstances positive reactions could be repeatedly produced for one member and not for another (Figs. 11 and 12). The *Daphnia* gathered from a casting pool in the early spring gave strong positive reactions not only to those sensitive to other *Crustacea,* but during the height of the grass pollen season *Daphnia* almost universally reacted on patients sensitive to grass pollens. The other *Crustacea* extracts did not react on these patients. This could not be accounted for on the basis of pollens in the water where the organisms were gathered, because the *Daphnia* was obtained before the grass or even the common trees were in active pollination. Recently, in autumn and winter months, this same *Daphnia* material reacted infrequently even in patients reacting strongly to pollens.

TABLE VI. COMPARISON OF REACTIONS TO INSECT ALLERGENS WITH REACTIONS TO COMMON INHALANTS

COMMON INHALANT		SCRATCH TESTING		INTRADERMAL TESTING			TOTAL TESTED
		NEG. TO INSECTS	POS. TO INSECTS	NEG. TO INSECTS	POS. TO INSECTS	NEG. TO BOTH	
House dust	Pos.	6	7	57	95	58	250
	Neg.	15	12	43	15		
Epidermals	Pos.	12	14	56	95	53	251
	Neg.	9	9	44	12		
Pollens	Pos.	13	13	35	68	71	253
	Neg.	8	9	63	44		

These observations are wholly without satisfactory explanation. On a recent December day that was free of pollen, *Daphnia* was again gathered and will be lyophilized and used in comparison, both clinically and biochemically, to determine whether there was a contaminant or a gradual alteration in the allergen itself by autolysis or other enzymatic action.

Reaction to salt water plankton (shrimp) almost invariably coincided with skin reactions to edible crab, shrimp, and lobster. The possibility that these minute but strongly allergenic organisms may affect ocean swimmers, such as *Daphnia* in the case of those swimming in ponds and pools,[3] has been alluded to by several observers[59] and may occur more frequently than we have previously supposed.

Brine shrimp eggs which were grown, defatted, and tested as a concentrated aqueous extract reacted less frequently than the hatched insects themselves or than pollens and other inhalants. This potential allergen may react more frequently, however, in populations living in areas where the tiny eggs may be part of the atmospheric dust. Such occurrences would be isolated problems and might be readily overlooked. It might be well to do more routine testing with an extract of shrimp eggs in those areas where this material may be present.

The relationship between skin reactions to insect allergens and to the more common inhalants led to several striking general observations. Patients who failed to react even by intradermal testing to any of the inhalants or ingestants rarely reacted to the insect allergens. For the most part, large immediate reactions occurred as often with insect allergens as with other allergens (Table VI).

In Table VII correlation of skin reactions to insect allergens with allergic disorders is roughly presented. The largest number of cases is represented by perennial allergy symptoms affecting the respiratory tract. Many of these patients also had pollinosis and dermatologic manifestations of allergy of secondary importance. The cases of pollinosis without other complicating allergic disorders were relatively few because studies were begun on these 252 patients in July, after the peak of our pollen season had passed. The group with uncomplicated dermatologic disorders were, in most cases, of the "atopic" form and also in most cases reacted to environmental allergens by scratch or intradermal testing. At this time, studies with the unprocessed insects or with their oily extracts by patch test had not been evaluated. This phase of study of insects as contactants will deserve, however, thorough study.

TABLE VII. INCIDENCE OF SKIN REACTIONS TO INSECT ALLERGENS IN RELATION TO ALLERGIC DISORDERS

ALLERGIC DISORDER	TOTAL	POSITIVE	NEGATIVE
Asthma, nonseasonal allergic rhinitis (with or without other complicating allergic disorders)	217	100	117
Pollinosis (without history of other allergic disorders)	15	8	7
Urticaria (without history of other allergic disorders)	3	1	2
Other dermatologic disorders* (without history of other allergic disorders)	16	6	10
Emphysema obstructive (without history of allergic disorders)	3	0	3

*Primarily eczema and eczematoid dermatitis in late youth and adulthood.

Skin reaction to silk, in the course of routine skin testing, is not uncommon. It occurs in a population that has been without the imported silk fabric for more than a decade. Reactions occur in children and young adults who have lived their days in a postwar environment of synthetics. Weiner[57] reports a case of bronchial asthma due to silk in a 6½-year-old girl. These were observations that aroused interest in insects as possible allergens to explain such frequent occurrences of silk sensitivity. Allergists have periodically commented on the

relationship of skin reactions to moth, silkworm, and silk. This is, indeed a frequent observation (Fig. 14). Table VIII shows a relationship that is not too reliable as a presumptive index of insect sensitivity. Strong reactions to

TABLE VIII. RELATED FREQUENCE OF REACTIONS TO INSECT ALLERGENS AND TO SILK

REACTION	NUMBER	PER CENT
Positive to insects and positive to silk	21	10
Positive to insects and negative to silk	86	37
Negative to insects and positive to silk	5	2
Negative to insects and negative to silk	118	50
Total	230	

insects have occurred with negative reactions to silk (Figs. 5 and 6). On the other hand, strong silk reactions have also been elicited with an absence of reaction to moth and any of the other insect allergens (Figs. 3 and 4). This can best be explained by the presence of several allergenically distinct substances and adds to the frustration of anyone seeking a common denominator.

TREATMENT WITH INSECT ALLERGENS

Eighty-three patients for whom insect allergens are included in the specific treatment were selected on the basis of (1) clinical history, (2) proportionately larger reactions to insect allergens than to any of the other inhalants, and (3) history of lack of adequate relief from treatment with conventional environmental allergens.

None of these patients is being treated exclusively with insect allergens. Results cannot be even grossly evaluated at this early date. An encouraging note is brought out in unsolicited reports by five such patients currently under treatment that for the first time their respiratory symptoms are well controlled. A good deal more observation and selection of patients will be required, however to justify our inclusion of this insect allergen in our routine treatment of allergic disorders.

SUMMARY

1. Insects as inhalant allergens fulfill the postulates set down for pollinating plants causing hay fever and asthma.

2. The nature of the insect allergens is not well understood; nor is there sufficient evidence to place the specificity at the level of species or higher in the table of classification.

3. The known protein chemistry of the insect cuticle is reviewed as it might be related to insect allergens and includes specific comments on chitin, arthropodin, and silk.

4. Insect materials for our studies were obtained by field and light trap "catches" and by breeding in the laboratory.

5. Handling of insect material is described, and the need for prompt processing or quick freezing is discussed.

6. The method of preparation of insect allergens at first varied greatly, but a standardized technique was established and is described in detail.

7. Clinical studies are tabulated on 252 consecutive unselected patients in relation to age, sex, race, geographic distribution, occupation, clinical diagnosis, and sensitivity to other inhalant allergens.

8. Treatment with insect allergens received only few brief comments, since it is too early to draw any conclusions on results.

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LOCAL ALLERGIC EDEMA INDUCED BY INJECTED PROCAINE

Diagnostic Value of the Twenty-Four-Hour Intracutaneous Test

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ALLERGY to procaine is uncommon. Its most frequent manifestation is contact dermatitis, occurring chiefly as an occupational disease among dentists.[1-3] Anaphylactic shock due to procaine is a rarity, perhaps best documented by Criep and Ribiero,[4] and also noted in the course of repeated intravenous treatments with this drug.[5] Others dispute these reactions and, indeed, appear to doubt that allergy to injected procaine occurs at all.[6, 7]

The present report concerns three cases in which there was repeated reaction to procaine, characterized by definite, even marked, swelling of the cheek and face at the site of injection for dental anesthesia. In each instance a preliminary impression of infection or trauma as a cause of the edema had to be discarded. The suspicion of allergy was confirmed by the demonstration of a positive skin test reaction of the delayed twenty-four-hour type to intracutaneous procaine. This form of procaine allergy appears to be largely ignored in most current discussions of hypersensitivity to this compound. Thus, neither Criep and Ribiero, in their classification of procaine allergy,[4] nor Sadove and associates,[8] in their review of reactions to local anesthetics, take note of it. Gray and Geddes[9] fail to refer to it in their extensive review of the local anesthetics. Rickles, however, has reported ten such cases, each with a positive skin test reaction of the twenty-four-hour type,[10] and Mitchell described two more, also with similar positive delayed skin reactions on test.[11] Adler and Simon's[12] earlier case differed in that it reacted not only with edema but with vesiculation as well and presented positive patch and intracutaneous skin test reactions to procaine.

THREE CASES OF EDEMA DUE TO PROCAINE

Case 1.—Patient F. K., a 42-year-old housewife, was seen in May, 1952, because of marked edema of the face appearing two to four hours after minor dental work done under procaine anesthesia. The swelling occurred on two occasions, lasting six to eight days each time, and was accompanied by noticeable itching. There was no evidence of any generalized reaction to the injection. Three years earlier she had noticed a slight similar reaction to a local anesthetic. She gave a history of a brief period of urticaria many years before, proneness to wheezing after colds, and some intolerance to milk, which caused diarrhea. Family history was positive for allergy.

Intracutaneous tests were carried out with 2 per cent procaine in aqueous solution. The immediate reaction was negative, but the delayed test done with 0.1 ml. was positive at twenty-four hours. Similar tests with 1 per cent Monocaine and 2 per cent Xylocaine

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likewise gave negative immediate reactions. However, the delayed twenty-four-hour test was even more strongly positive to Monocaine, although negative to Xylocaine. To confirm further the significance of these positive responses, the three anesthetics were injected subcutaneously into the outer upper arms. Local edema was definitely visible twenty-four hours later at the procaine and Monocaine sites but not at the site of Xylocaine injection. These reactions reproduced the local swelling which had been the patient's chief complaint. The patient's dentist was advised that Xylocaine should be well tolerated. This proved correct, as subsequent procedures were performed under Xylocaine anesthesia without untoward incident.

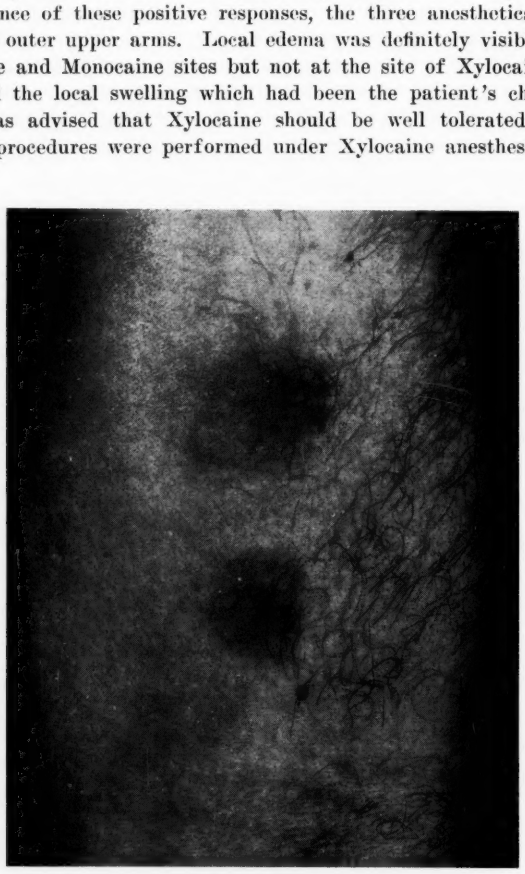

Fig. 1.—Case 2. Positive twenty-four-hour skin test reactions to procaine (upper) and Monocaine (lower) in patient with local allergic edema due to procaine.

Case 2.—M. G., a 26-year-old female schoolteacher, was seen in April, 1955, with a history of marked swelling over the jaw which occurred on three occasions after minor dental procedures under procaine anesthesia. The last time almost the entire left side of the face had been affected, with partial closure of the eye due to lid edema. Twice the reaction lasted for five days, and on the third occasion it took two weeks for the edema to subside. There was no itching but some local pain was present, aggravated by chewing and eating. There was no indication of any generalized reaction. She gave no history of atopy. Two years earlier, following penicillin injections, she had persistent generalized pruritus without eruption for several weeks. Family history revealed that the patient had a maternal aunt with severe asthma. Members of the immediate family were free of clinically apparent atopy.

Intracautaneous tests were carried out with 2 per cent procaine, 1.5 per cent Monocaine, 2 per cent Metycaine, 2 per cent Pontocaine, 2 per cent Unacaine, 1 per cent Lucaine, 1 per cent Cyclaine, and 2 per cent sodium sulfadiazine in aqueous solution. Several of these which were available without epinephrine could be read for the immediate reaction, and all were negative. At the twenty-four-hour reading procaine, Monocaine, and Unacaine

gave positive tests consisting of a circular area of erythema about 3.0 cm. in diameter with some central edema (Fig. 1). In this instance also, Xylocaine was recommended as a safe substitute for procaine and proved to be well tolerated in the course of subsequent dental procedures.

Because of the history of reaction to penicillin, this patient was also tested with crystalline penicillin G, 20,000 units per milliliter. The immediate test was negative by both scratch and intracutaneous methods. The test for the delayed reaction, after intracutaneous injection of 0.1 ml. of penicillin solution, proved positive at twenty-four hours, with an area of pink erythema 3.0 cm. in diameter and central edema. This induced lesion persisted longer than the reaction to procaine; it did not clear entirely within eight days. It was accompanied by more marked pruritus than the positive test lesions to the local anesthetics. Routine allergy tests with pollens and inhalants were negative.

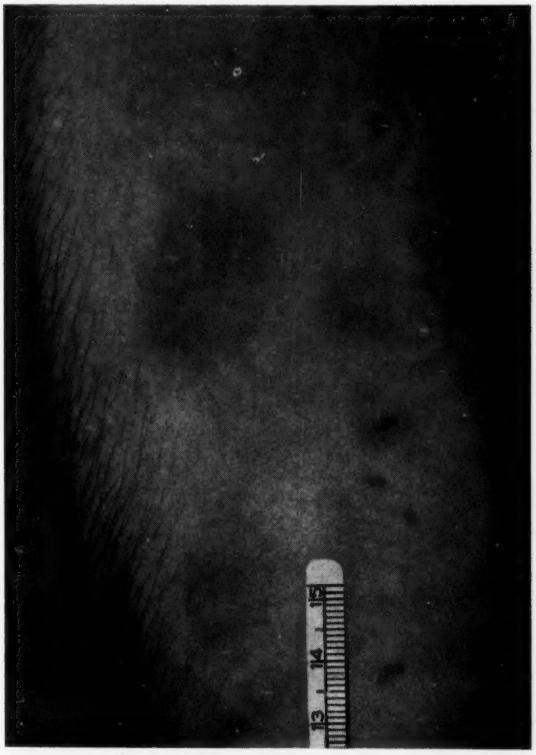

Fig. 2.—Case 3. Positive twenty-four-hour skin test reactions to procaine (left upper) and crystalline penicillin G (left lower) and slightly positive reaction to Monocaine (right). Negative test to Xylocaine (right lower). Case of local allergic edema due to procaine.

In November, 1957, two and one-half years after first observation, this patient was retested with procaine, Monocaine, and crystalline penicillin G solutions as she had been previously. Once again she reacted positively to all three but most strikingly to penicillin. Her allergy to these substances thus proved to be persistent. Two weeks after the retests, serum was tested for antibodies. No precipitation to either crystalline penicillin G or procaine could be demonstrated by either the gel diffusion technique or the ring method. Complement-fixation antibodies to these substances were also absent.

Case 3.—K. D., a 23-year-old female hospital worker seen in September, 1957, had noted in the preceding three months that three times she had developed swelling of the cheek on

the side of minor dental work done under procaine anesthesia. This began twelve to fourteen hours after treatment and lasted thirty-six to forty-eight hours. There was neither pain nor itching. Her reaction was thus less intense than that of the two other patients. Again there was no sign of general reaction. She had had procaine many times previously without ill effect. She was prone to an occasional single urticarial wheal, but there was no other personal history of allergy. Family history was negative for allergy. One year before she had received twice-daily injections of procaine penicillin for treatment of pneumonia, but there had been no evidence of any allergic reaction.

Intracutaneous tests were done with 2 per cent procaine. The immediate reaction was negative. The delayed test, after injection of 0.1 ml., was positive at twenty-four hours. The reaction at the test site began at four hours. It subsided after its peak at twenty-four hours, clearing almost completely by the end of forty-eight hours. It was characterized by a circular zone of bright pink erythema with central infiltration. A similar, though less intense, reaction to 1 per cent Monocaine was observed (Fig. 2). Negative tests were obtained with 2 per cent Xylocaine, 1 per cent Pontocaine, and 1 per cent Unacaine. This patient, too, was found able to tolerate Xylocaine anesthesia for subsequent dental procedures.

Because of the positive reaction to penicillin obtained in Case 2, skin tests with aqueous solution of crystalline penicillin G, 20,000 units per milliliter, were carried out. The immediate reaction was negative, but the delayed test reaction elicited with 0.1 ml. was definitely positive, presenting a circular zone of erythema, 2.0 cm. in diameter, with slight central edema.

Routine allergy tests with pollens, inhalants, and foods were negative throughout. This patient's serum was tested for antibodies in November, soon after retest had shown persistent positive skin test reactions to procaine and penicillin. No evidence of precipitins to procaine or to penicillin could be demonstrated by gel diffusion or ring techniques, nor were complement-fixing antibodies present. Passive transfer tests were negative to procaine and penicillin, observation being made for both immediate and delayed reactions.

CONTROL GROUP

Sixty-five adult ward patients, both men and women, were skin-tested with 2 per cent aqueous procaine solution. Twenty-five were tested intracutaneously for the immediate reaction, and all had negative reactions. All sixty-five were tested with 0.1 ml. intracutaneously for the twenty-four-hour test, and again all gave negative responses.

TWO CASES OF PENICILLIN ALLERGY WITH POSITIVE PROCAINE SKIN TESTS

Case 4.—M. B., a 24-year-old woman, had urticaria for seven months, occurring two months after treatment with procaine penicillin. When the patient was seen in May, 1955, skin tests with crystalline penicillin G, 30,000 units per milliliter, were negative on immediate reading, but the 24- to 48-hour reaction was positive. This showed a strongly positive reaction of erythema and central edema. Similar tests with 2 per cent aqueous procaine gave a negative immediate reaction and a milder but definitely positive twenty-four-hour skin test reaction (Fig. 3).

Case 5.—E. L., a 45-year-old man, first gave evidence of allergy to penicillin in 1953, when he reacted with marked erythema of the groins and buttocks twenty-four hours after a single penicillin injection for acute tonsillitis. He was skin-tested in 1954 with crystalline penicillin G, 10,000 units per milliliter, and gave a strongly positive delayed reaction, with erythema and edema; this reaction reached its height at forty-eight hours, but lasted with some eczematization for several days. A positive skin reaction to penicillin could be obtained with as little as 10 units per milliliter. The patient was not tested with procaine until January, 1958, when he was shown to have a negative immediate test but a positive delayed test reaction with definite erythema and edema, more than 2.0 cm. in diameter. This test lesion began to fade after forty-eight hours.

DISCUSSION

The phenomenon of local allergic edema due to injected procaine is not widely recognized. Although procaine has been in use for over half a century, reports of this type of allergic reaction have appeared only in recent years. It is possible that such reactions may be more frequent because of the widespread use of procaine penicillin since its introduction in 1948. In 1952 it was estimated that 75 per cent of the total production of penicillin was in the form of the procaine salt.[13] This slowly absorbed depot preparation contains procaine in 12 per cent suspension and may be a more potent sensitizer than the usual procaine solution. This possibility is suggested by the concomitant presence of a positive penicillin skin test of the twenty-four-hour type in two of the foregoing cases of procaine edema. Of interest also are two instances in which patients with penicillin allergy, who gave positive skin test reactions to penicillin, were shown to have a positive twenty-four-hour skin test reaction to procaine as well.

Fig. 3.—Case 4. Positive twenty-four-hour skin test reactions to crystalline penicillin G (upper) and to procaine (lower). Reactions at left are at sites negative at the fifteen-minute reading, induced with 0.02 ml. Case of seven months of urticaria after procaine penicillin.

The association of penicillin and procaine allergy requires clarification. It may represent coincidence, a susceptible patient reacting to each allergen independently. Cross-sensitization between the two compounds appears unlikely in view of their quite different chemical configuration. The more complex penicillin molecule does not break down into elements related to procaine. Against cross-sensitization, too, are observations of contact allergy to procaine

from procaine penicillin without allergy to penicillin.[14, 15] More complex explanations involving the possibility that penicillin allergy, with a positive skin test of the delayed type, may predispose to this form of procaine allergy are at present speculative.

In all three cases of procaine edema reported here, a positive skin test reaction was also elicited with Monocaine. Previous exposure to Monocaine may have occurred, for example, in Case 1 in which the skin test reaction to Monocaine was stronger than that to procaine. These two anesthetic compounds are chemically very similar, and both are esters of para-aminobenzoic acid. Included in the so-called procaine group of anesthetics are also Pontocaine, Butyn, Larocaine, and tutocaine. Cross-sensitization is considered more likely among members of this group on the basis of clinical observation,[1] in part confirmed by animal experiments.[16]

In Case 2 a positive skin test was found to Unacaine, a compound in which the amino group attached to the phenol ring is at the meta rather than the para position. This is in contrast with the observation by Rothman and colleagues that in contact dermatitis due to procaine, transfer of this amino group away from the para position is likely to be associated with loss of sensitivity.[1]

Xylocaine is sufficiently distinct in chemical structure so that cross-sensitization is unlikely. Each of the three patients with procaine edema was found to tolerate Xylocaine without difficulty. As a widely accepted anesthetic agent in both dentistry and surgery, this drug is at present the most likely safe substitute in the presence of procaine allergy. This is in accord with Rickles' observations.[10]

With the establishment of local allergic edema due to procaine as a definite entity, the warning might be justified that within the closed space of the spinal canal even a transient allergic reaction might prove a significant hazard. Anesthetics other than procaine might therefore be considered safer for intraspinal use.

SUMMARY

1. Three cases of repeated edema at the site of procaine anesthesia for minor dental procedures are described.

2. Marked swelling of the cheek and face was noted several hours after injection and lasted for two to fourteen days. There was no evidence of general reaction or urticaria.

3. A positive twenty-four-hour skin test reaction of erythema and edema was induced by the intracutaneous injection of procaine and by injection of Monocaine as well. Immediate skin tests were negative.

4. Skin tests with Xylocaine were negative. This anesthetic proved a safe substitute for procaine.

5. A positive twenty-four-hour skin test reaction to crystalline penicillin G was also present in two cases. Two other patients with clinical allergy to

penicillin also proved skin test-positive to procaine. A possible etiological relationship of procaine penicillin treatment to this form of procaine allergy is discussed.

The author is indebted to Dr. Liselotte Graf for carrying out the complement-fixation tests and to Jerome S. Mittelman, D.D.S., for his interest in this problem.

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DERMATITIS MEDICAMENTOSA APPARENTLY DUE TO TETRACYCLINE

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COMMERCIAL preparations of tetracycline (Achromycin, Tetracyn, Polycyline) were released for general usage in 1953, although chemically related antibiotics, such as chlortetracycline (Aureomycin) and oxytetracycline (Terramycin), were widely prescribed for many years previously. There are many reports about side effects and complications related to the last two drugs. Allergic reactions attributed to them are relatively rare. There are only very few reports of hypersensitivity to tetracycline, and we therefore believe that the following case might be of general interest.

CASE REPORT

The patient is a 35-year-old married housewife. She has a 9-year-old daughter who, at the age of 2 to 3 years, used to have recurrent urticaria attributed to strawberries. There were no other allergic manifestations in the family.

Past History.—The patient was always in good health except for recurrent toothache, for which she used to take preparations made up of aspirin, phenacetin, caffeine, and codeine, without unusual effect. In 1949, following a gynecologic intervention, she received penicillin injections. Penicillin tablets were prescribed for a pharyngitis in 1952. There was no allergic reaction. In January, 1955, the patient suffered from acute otitis media; she received penicillin injections, but when there was no improvement injections of streptomycin were added, and later Achromycin was given orally with good results. There were no allergic phenomena.

Present Illness.—On Nov. 25, 1955, following a febrile disease of two days' duration, her family physician diagnosed a "flu" and prescribed Achromycin capsules, 250 mg. each. The patient took one in the morning and one at noontime. In the evening a pink rash appeared on the upper extremities, associated with a temperature of 40° C. The Achromycin was discontinued and antihistaminics were prescribed. During the following week the patient's condition became worse; the rash spread and became more intense, she complained of pain in the large joints, and the fever remained at high levels. The patient denied any "fever" or "rash" in her immediate family or other contacts preceding the onset of her eruption.

On her admission to the hospital on Dec. 2, 1955, the patient complained strongly of severe pains in the large joints, headache, and generalized itching.

Examination revealed a maculopapular pink and almost hemorrhagic rash involving the entire body, including the face and extremities. The lesions were discrete, but at some sites they became confluent. The face was swollen and the mucous membrane of the mouth and tongue was involved and swollen (Fig. 1). No other abnormalities were revealed by the physical examination.

Laboratory Data.—In the blood there were 3,580,000 erythrocytes, 192,000 thrombocytes, and 12,000 leukocytes per cubic millimeter. There was a shift to the left in the

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differential count, with 7 per cent eosinophils; electrolytes, proteins, and bleeding and clotting times were normal. The erythrocyte sedimentation rate was 20 mm. in one hour and 50 mm. in two hours (Westergren). No L.E. cells were found. Routine examinations of urine and stools were normal.

Fig. 1.—Patient before treatment.

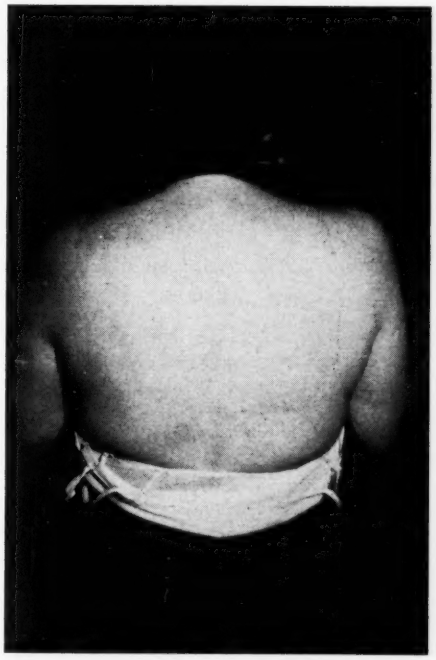

Fig. 2.—Patient after treatment.

Course.—A diagnosis of dermatitis medicamentosa due to Achromycin was suggested, and the patient was given daily intravenous drip injections of 20 I.U. ACTH in 500 c.c. of 5 per cent glucose solution. Following a few such treatments there was a marked improvement, the rash decreased, fever came down, and the white count and eosinophils

became normal. The dose of ACTH was gradually decreased and was discontinued when the pink discoloration of the skin disappeared and only desquamation and hyperpigmentation remained. The patient was discharged on the thirteenth hospital day in very good condition (Fig. 2).

Follow-Up.—The patient returned for a checkup in March, 1957, more than a year after her illness. She had no complaints except for her usual toothache and headaches, for which she used her usual preparations.

By this time an allergy clinic had been established at the hospital, and the patient was submitted to an allergic work-up. Routine intracutaneous tests were negative. We refrained from doing any direct testing with Achromycin lest a serious reaction develop. A passive transfer (Prausnitz-Küstner) was negative to Achromycin. An attempt to perform a collateral passive transfer[1] was made. Following the technique of Walzer,[2] serum of the patient was injected intracutaneously to persons who were given Achromycin or Terramycin orally; there was no local reaction.

DISCUSSION AND COMMENT

We were convinced by the history and course that the patient suffered from an allergic reaction to Achromycin. She received the drug for the first time, without undue effect, about one year before her present illness. The rash started on the day she received 500 mg. Achromycin for the second time. The patient apparently is not an atopic person, but this does not exclude the possibility of hypersensitivity to drugs. We have no positive direct proof of her sensitivity to Achromycin, since no cutaneous tests were done and no oral test dose was given. A negative skin test would not help us one way or the other. As for direct and collateral passive transfer, it should be emphasized that the serum was taken more than a year after the acute allergic reaction; even if this phenomenon was associated with the production of transferable reagins, it can be argued that the titer was lost with elapse of time.

In the medical literature at our disposal there are only two reports of allergic reactions to tetracycline. Sakamoto[3] described an anaphylactoid reaction following an intramuscular injection of 100 mg. tetracycline hydrochloride with procaine to a 43-year-old man. The patient received this medication for the first time, but he had previously received others. A skin test with procaine was positive and the author could not state definitely whether the allergic reaction was due to the tetracycline or to procaine. Welsh[4] described a patient who suffered from a fixed drug eruption due to chlortetracycline (Aureomycin) and crossed fixed drug eruption at the same sites, due to oxytetracycline (Terramycin). Some time later he received tetracycline (Achromycin) and again a rash appeared at the same sites. Since the tetracycline ring is common to these three drugs, the author believed that it was responsible for the reaction. If this supposition is proved, the chances are that in the near future we may encounter more allergic phenomena after administration of tetracycline to patients who had previously received oxytetracycline and/or chlortetracycline.

SUMMARY

A 35-year-old nonatopic woman was treated with penicillin, streptomycin, and Achromycin for otitis media. One year later she was given two capsules

of Achromycin, 250 mg. each, for a short febrile illness. On the same day the patient developed an intense itching, an extensive rash, and fever. These progressed in spite of antihistaminics and within one week formed maculopapular single and confluent lesions covering the entire skin, involving the mucous membrane of the mouth and tongue, and associated with marked pain in the large joints. She responded well to intravenous ACTH.

An allergy work-up performed one year later revealed a negative collateral and direct passive transfer to Achromycin. Because of the risks involved, no testing with Achromycin was done on the patient directly. Nevertheless, on the basis of the history and the course of the disease, it is considered likely that the patient's dermatitis was an allergic reaction to tetracycline.

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EXPERIMENTAL EOSINOPHILIA

Studies on the Origin and Relationship of Tissue Eosinophil Cells to Peripheral Blood Eosinophilia

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INTRODUCTION

THE association of the eosinophilic leukocyte with in vivo reactions of hypersensitivity, allergic phenomena, and foreign protein response is a familiar and accepted picture, but the exact circumstances surrounding the true origin of this cell and its evolution and function are still poorly understood. Discussions of controversial theories and a considerable amount of clinical and experimental evidence in this regard may be found summarized in several comprehensive reviews and reports.[1-6]

Although the exact function of the eosinophil is not known, experimental evidence has demonstrated its productive response to many and varied stimuli. Early attempts at inducing experimental eosinophilia included pilocarpine injections,[7] protein feedings,[8] experimentally induced parasitic infections,[9] injections of parasitic extracts,[10] hemolytic sera,[11] foreign erythrocytes[12] and proteins,[13] anaphylactic reactions,[14] and drainage of sites of bacterial infection.[13] The eosinophilic granule has been thought to contain iron,[15] phospholipid,[16] a protein center,[17] and desoxyribonucleic acid.[18] High on the list of substances known to be chemotactic for eosinophils are foreign proteins, antigens, antibodies, destruction products of lymphocytes[2] and histamine.[6] Ringoen[1] felt that foreign proteins were the chief factors responsible for increased eosinophil production. On the other hand, the studies by Campbell, Drennan, and Rettie[19] and those by Campbell[20] point out the intimate relationship of eosinophil response to conditions associated with anaphylaxis and suggest its dependence upon hypersensitivity mechanisms. In further consideration of responsible factors of sensitization that might be related to the production of eosinophilia, there are other reports that suggest (1) the importance of the reaction of a sensitized organism to specific antigen rather than to the simple presence of an antigen per se,[21] (2) an eosinotactic stimulus furnished by release of an agent as a product of the antigen-antibody reaction or by one of the enzymes activated through such reaction,[22] (3) the involvement of eosinophils in antibody production or release,[23] (4) the possibility that eosinophils may perhaps

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even form the enzymatic template which is phagocytized and utilized by mononuclear cells in antibody formation,[24] (5) and the role played by eosinophils in the carrying of anaphylactogenic material.[25] However, the work of Redd and Vaughn[26] suggests that the eosinophilia of anaphylaxis is not dependent upon active antibody formation. Code[27] and Vaughn[6] believe that eosinophilic granulocytes are the chief carriers of histamine which may have its source or release at the site of in vivo antigen-antibody union, and it has even been suggested that eosinophils contain antihistamine activity.[28] In marked contrast is an opinion advanced by Godlowski[29] that eosinophilia is a manifestation of "vagal reaction."

Circumstances surrounding hematopoiesis of the circulating eosinophilic granulocyte are also imperfectly understood. It has been generally accepted that the eosinophil, along with other members of the granulocytic series, has its origin in the extravascular myeloblast with the development of specific granules at the premyelocyte stage. These cells then progress through myelocytic and metamyelocytic stages until the definitive eosinophilic granulocytes find their way into the circulation. Duran-Jorda,[30, 31] on the basis of original studies, proposed that eosinophils are derived from lymphocytes in gastric and intestinal mucosa via intermediary Paneth cells whose eosinophilic granules are preformed red cells, that eosinophils migrate from the mucosa to other tissues where they burst and liberate the red cells, and that the mononuclear residual of the eosinophil is the lymphocyte which eventually returns to the gastrointestinal mucosa to repeat the cycle.

The origin of tissue eosinophils also poses a controversial problem. This question has been reviewed and summarized by Biggart,[32] and more recently it has been studied by Samter and his associates.[4, 33] Tissue eosinophils have been considered to originate from parent cells of various sources,[1] such as lymphocytes, large mononuclear cells, plasma cells, and adventitial cells, and some experimental studies also seem to indicate a clasmatocytic origin. However, Homma[34] felt that there were no intermediary stages existing between small lymphocytes, large lymphocytes, and macrophages on one side and eosinophils on the other. Whether eosinophilic leukocytes migrate from the bone marrow via the peripheral blood circulation into tissue areas or whether these cells migrate out of tissue sites of formation into the peripheral vascular system has never been conclusively demonstrated. It is entirely possible that both of these mechanisms may be in operation. Maximow[35] regarded eosinophils of connective tissue, excluding only those of intestinal mucosa, as eosinophilic leukocytes that had migrated from the blood stream, and Samter[3, 4] has offered further evidence in favor of a peripheral blood origin of tissue eosinophils. Clinical observations in pathologic states have suggested a possible difference between peripheral blood and tissue eosinophils. Cooke[36] noted discrepancies between the relative number of eosinophils in blood and tissues, and Rappaport[37] observed morphologic differences in the cell nuclei of the peripheral blood eosinophil and its histologic counterpart in allergic nasal mucosa.

In a previous report[38] we have demonstrated the lack of dependence of tissue leukocytic and eosinophilic cellular components of experimentally produced microscopic lesions of arterial sensitization upon their hematologic circulating cellular counterparts. In the present study it has been our purpose to examine further possible sources of tissue eosinophils and specifically their relationship to eosinophilic granular leukocytes found within the peripheral circulation and in such hematopoietic organs as bone marrow and spleen. This problem has been approached experimentally by employing rabbits whose circulating blood and bone marrow had been rendered leukopenic and eosinopenic by treatment with nitrogen mustard. The production of cutaneous and subcutaneous eosinophilic cellular infiltration through a foreign protein response was then attempted with the localized introduction of heterologous species liver extract into the tissue under study.

EXPERIMENTAL PROCEDURE

Albino rabbits of the New Zealand strain, ranging in weight from 1.5 to 2.5 kilograms were used as experimental animals throughout this study. These rabbits were divided into three experimental groups of six each. Crude beef liver extract* assayed at 2 meg. of cyanocobalamin per milliliter was administered to all animals of the three groups by a series of alternating intramuscular, intraperitoneal, and intravenous injections. A total weekly dose of 5.5 ml. was equally divided over the period of six days. Aluminum hydroxide gel, 0.25 ml., was added to each dosage of liver extract that was injected into the gluteal muscles. Nitrogen mustard (methyl bis-beta chlorethylamine)† injections in a dosage of 1 mg. per kilogram of body weight were given intravenously every third day into the marginal ear veins of the animals in two of the experimental groups.

Initial total eosinophil counts, together with total white and differential blood cell counts, were determined prior to initial injections of beef liver extract and/or nitrogen mustard. These were repeated at weekly intervals thereafter and again immediately prior to the sacrifice of each animal. Propylene glycol solution containing 0.1 per cent phloxine was employed in total eosinophil counts according to the technique described by Pilot.[39]

In order to minimize confusion with pseudo-eosinophils[40, 41] known to occur in the rabbit, all tissue eosinophils were carefully studied in order to identify the characteristic eccentric, bilobed, plump, round, blue-staining nucleus and the characteristic coarse, red, cytoplasmic granules. Possible error in identifying peripheral blood eosinophils was also minimized by utilizing the chamber method of Pilot[39] in preference to the conventional differential stained smear for counting these cells.

*Supplied by Armour Laboratories, Kankakee, Illinois, through the courtesy of L. W. Van Ness and by Pitman-Moore Company Division of Allied Laboratories, Indianapolis, Indiana, through the courtesy of Dr. Philip A. Boyer, Jr.

†Supplied by Merck Sharp and Dohme, Rahway, New Jersey, through the courtesy of Dr. Frederick K. Heath.

Animals of all three groups received intradermal injections of 0.75 ml. of crude beef liver extract plus 0.25 ml. aluminum hydroxide gel into the dermis at a dorsolateral site midway between the pelvic and pectoral regions. Skin biopsy specimens of these locally injected sites were taken forty-eight hours later and prepared for microscopic study.

All animals of the three experimental groups were sacrificed by air embolism, and sections of spleen, along with the local liver-injected skin sites, were taken and prepared for microscopic study according to standard histologic techniques. Marrow smears were prepared from the femoral bone and treated with Wright-Giemsa stain for differential cell study.

Prior to and following the completion of injections of beef liver extract and/or nitrogen mustard, each animal was bled from the central artery of the ear and the serum was tested for the presence of circulating antibodies to beef liver. This was done according to two different techniques: (1) the "ring test" in which the development of an antigen-antibody reaction was indicated by the formation of precipitate at the interfacial junction of the beef extract dilution layered over the rabbit serum and (2) the agar gel diffusion plate technique described by Ouchterlony[42] in which the antigen-antibody reaction was indicated by a band of precipitate developing in a 1 per cent agar-filled Petri dish through which beef liver extract and rabbit serum diffused from oppositely placed wells within the agar.

Group I.—Each animal received injections of only the beef liver extract for a period of six weeks. Intradermal injections of liver extract, with subsequent skin biopsy, were completed at the end of the third week and again later at the end of the sixth week, forty-eight hours prior to sacrifice.

Group II.—Each animal received injections of liver extract for a total of six weeks. During the fifth and sixth weeks, however, in order to effect a leukopenia, nitrogen mustard was also administered with the beef liver extract. Intradermal injections of liver extract, with subsequent skin biopsy, were given at the end of the third week, just prior to the administration of nitrogen mustard, and were repeated again in the sixth week, forty-eight hours prior to sacrifice.

Group III.—Each animal received only nitrogen mustard for two weeks in order to induce a preliminary leukopenia. This was followed by the continued administration of nitrogen mustard plus beef liver extract for an additional two-week period. Intradermal injections of liver extract with subsequent skin biopsy were completed at the end of the second week, just prior to the administration of liver extract, and again at the end of the fourth week, forty-eight hours prior to sacrifice.

RESULTS

Group I (Receiving Only Liver Extract).—

Peripheral blood counts: All six animals in this group developed a maximum elevation of peripheral blood eosinophils by the end of the third week

of beef liver extract injections. The number of circulating eosinophils increased to an average value of 2,468 cells per cubic millimeter above the initial average cell count of 640 eosinophils per cubic millimeter. With continued injections of beef liver extract during the fourth, fifth, and sixth weeks, there was no further increase in circulating eosinophils, and thereafter a slight decrease occurred (Table I, Fig. 1). There was a parallel rise in the total number of circulating leukocytes during the first three weeks of treatment with liver extract, and there were some fluctuations during the remaining three weeks (Table I, Fig. 2).

TABLE I. TOTAL CIRCULATING EOSINOPHIL AND WHITE BLOOD CELL COUNTS OF THE SIX RABBITS OF GROUP I, TREATED WITH CRUDE BEEF LIVER EXTRACT

ANIMAL NO.	BEFORE TREATMENT	1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK	5TH WEEK	6TH WEEK
			Eosinophils per Cubic Millimeter				
1 A	499	899	3,252	3,870	3,370	2,420	3,373
2 A	699	1,209	1,465	2,200	2,076	1,854	1,698
3 A	777	1,320	3,164	3,807	2,786	2,442	2,209
4 A	666	1,010	1,054	1,721	1,531	1,043	733
5 A	555	1,099	1,132	1,687	2,375	1,610	1,221
6 A	644	1,199	1,232	1,521	1,477	3,630	1,188
	Mean:						
	640	1,123	1,883	2,468	2,269	2,133	1,737
			Leukocytes per Cubic Millimeter ($\times 10^{-3}$)				
1 A	15	17	23	27	21	15.5	19.3
2 A	12	12.6	15.5	12.1	11.4	10.6	15.3
3 A	12.5	14	18.5	23.9	19.3	12.4	14.4
4 A	13.4	16	20	19.5	15.5	17.9	17.5
5 A	18.2	17	13	19.7	15	14.2	20.5
6 A	9.6	12.7	13.8	16.4	14	28	17
	Mean:						
	13.5	14.9	17.3	19.8	16	16.4	17.3

Each animal received injections of only the beef liver extract for six weeks.

Skin sections: Microscopic examination of the skin sites locally injected with liver extract and biopsied at the end of the third week exhibited a localized infiltration of granular eosinophilic cells into the epidermis and corium. This finding was again noted in the skin sections taken three weeks later, just prior to sacrifice. This eosinophilic cellular infiltration was usually associated with an interstitial tissue edema (Fig. 3, *B*).

Spleen: Histologic sections of the spleen also demonstrated a microscopic tissue eosinophilic granular cell infiltration throughout the splenic parenchyma, with some associated interstitial tissue edema (Fig. 3, *C*).

Bone marrow smears: The differential cell study of the femoral bone marrow revealed a few mature lymphocytes, an increased number of myelocytes, an average number of polymorphonuclear neutrophilic leukocytes, and a marked increase of granular eosinophilic cells predominating in all six animals (Fig. 3, *A*).

Antibody determinations: The development of circulating precipitating antibodies to beef liver extract was demonstrated in the sera of all six animals after completing series of beef liver injections.

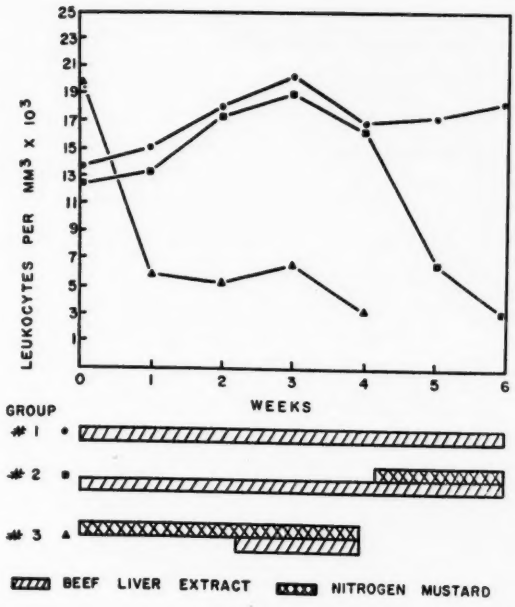


Fig. 1.—Graph representing mean circulating eosinophil counts of rabbits of Group I (treated with liver extract), Group II (treated initially with liver extract, followed by the simultaneous administration of liver extract and nitrogen mustard), and Group III (treated initially with nitrogen mustard, followed by the simultaneous administration of nitrogen mustard and liver extract).

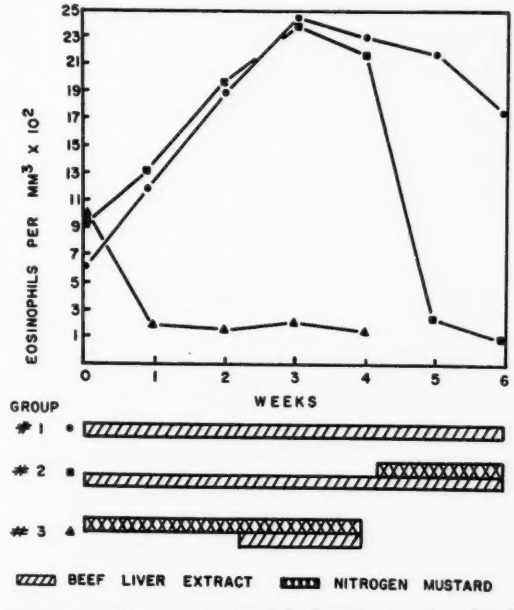


Fig. 2.—Graph representing mean total leukocyte counts of rabbits of Group I (treated with liver extract), Group II (treated initially with liver extract followed by the simultaneous administration of liver extract and nitrogen mustard), and Group III (treated initially with nitrogen mustard, followed by the simultaneous administration of nitrogen mustard and liver extract).

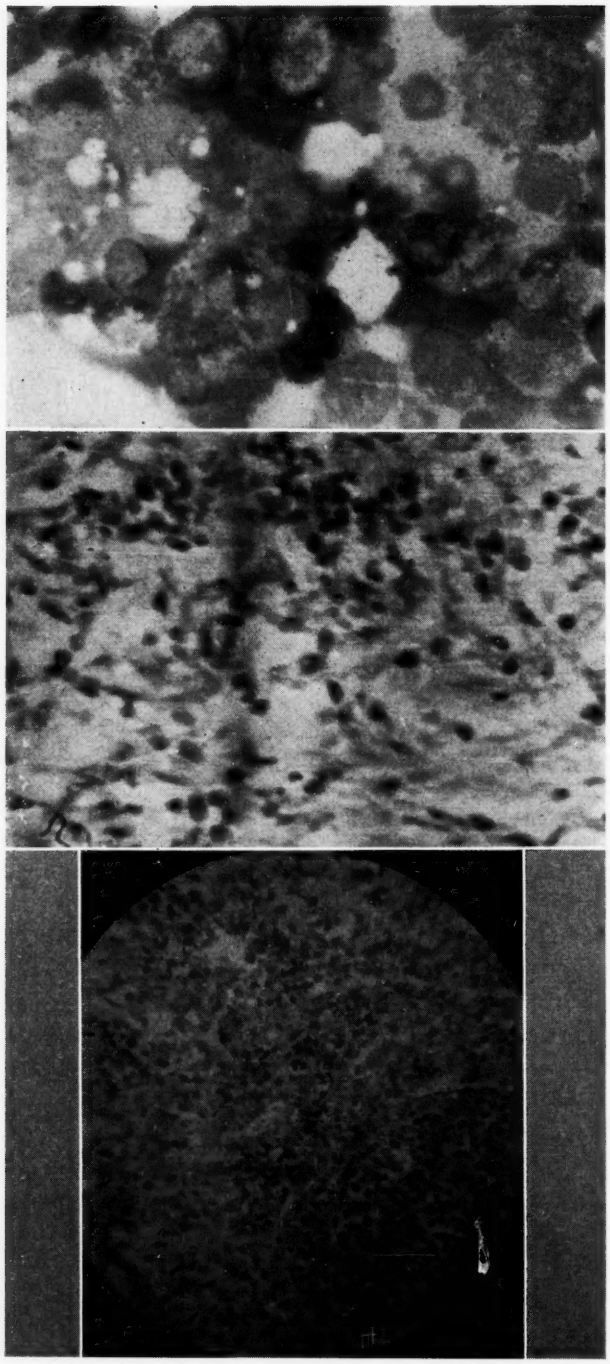


Fig. 3.—Tissue sections from a rabbit that had received injections of crude beef liver extract for a period of six weeks. *A*, Femoral bone marrow smear showing a marked predominance of eosinophilic granular cells and extracellular eosinophilic granules throughout; *B*, skin site that had been injected locally with crude beef liver extract forty-eight hours previously, showing a diffuse infiltration of eosinophils throughout the corium and an associated interstitial edema; *C*, spleen showing an infiltration of eosinophils throughout the splenic parenchyma.

Group II (Receiving Beef Liver Extract for Six Weeks Plus Nitrogen Mustard During the Fifth and Sixth Weeks).—

Peripheral blood eosinophilia was effected in all six animals during the first three weeks of beef liver extract injections, with an average rise of from 949 eosinophils per cubic millimeter to 2,438 eosinophils per cubic millimeter. With continued administration of the liver extract, no further cell rise was noted. With the concomitant administration of nitrogen mustard during the fifth and sixth weeks, however, the total number of peripheral blood eosinophils decreased to an average value of 59 eosinophils per cubic millimeter (Table II, Fig. 1). The total number of circulating leukocytes exhibited a parallel but lesser increase from an average value of 12,450 white blood cells per cubic millimeter to 18,217 white blood cells per cubic millimeter during the first three weeks of liver administration. After a slight fluctuation during the following week, a marked leukopenia averaging 2,650 white blood cells per cubic millimeter developed after two weeks of nitrogen mustard treatment associated with the liver extract administration (Table II, Fig. 2).

TABLE II. TOTAL CIRCULATING EOSINOPHIL AND WHITE BLOOD CELL COUNTS OF THE SIX RABBITS OF GROUP II, TREATED INITIALLY WITH LIVER EXTRACT, FOLLOWED BY THE SIMULTANEOUS ADMINISTRATION OF NITROGEN MUSTARD AND LIVER EXTRACT

ANIMAL NO.	BEFORE TREATMENT	1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK	5TH WEEK	6TH WEEK
			Eosinophils per Cubic Millimeter				
1 B	1,554	1,832	3,441	3,818	3,352	67	67
2 B	1,088	1,487	1,854	2,753	2,553	167	122
3 B	666	944	1,943	2,161	2,061	78	67
4 B	599	1,476	1,310	1,454	1,543	111	33
5 B	688	810	1,354	1,388	1,299	311	22
6 B	1,099	1,265	1,832	3,053	3,053	544	44
	Mean:						
	949	1,302	1,956	2,438	2,162	213	59
			Leukocytes per Cubic Millimeter ($\times 10^{-3}$)				
1 B	18	18.2	27	24	21.8	3.8	2.8
2 B	13	14	15	23.8	16.9	3.1	3.1
3 B	12	10.5	16.5	14.9	14.5	3.4	3.4
4 B	11	9.8	11.7	10.6	10.6	6.9	2.2
5 B	9.5	9	13.8	17	14.2	7.4	1.5
6 B	11.2	18	17.7	19	19	6.2	2.9
	Mean:						
	12.5	13.3	17	18.2	15.6	5.1	2.7

Each animal received injections of liver extract for a total of six weeks. During the fifth and sixth weeks nitrogen mustard was administered along with liver extract.

Skin sections: Histologic study of the biopsied sections of the locally injected skin sites at the end of the third week demonstrated a localized infiltration of eosinophilic granulocytes into the epidermis and corium with an associated interstitial edema in all animals. At the end of the sixth week following the induced circulating eosinopenia, and just prior to sacrifice, a similar histologic picture was seen in the liver-injected skin sites (Fig. 4, *B*) although the eosinophilic infiltration was somewhat milder in two of the six animals studied.

Spleen: In sections of the spleens studied microscopically after sacrifice, only occasional eosinophilic granulocytes were noted scattered throughout the

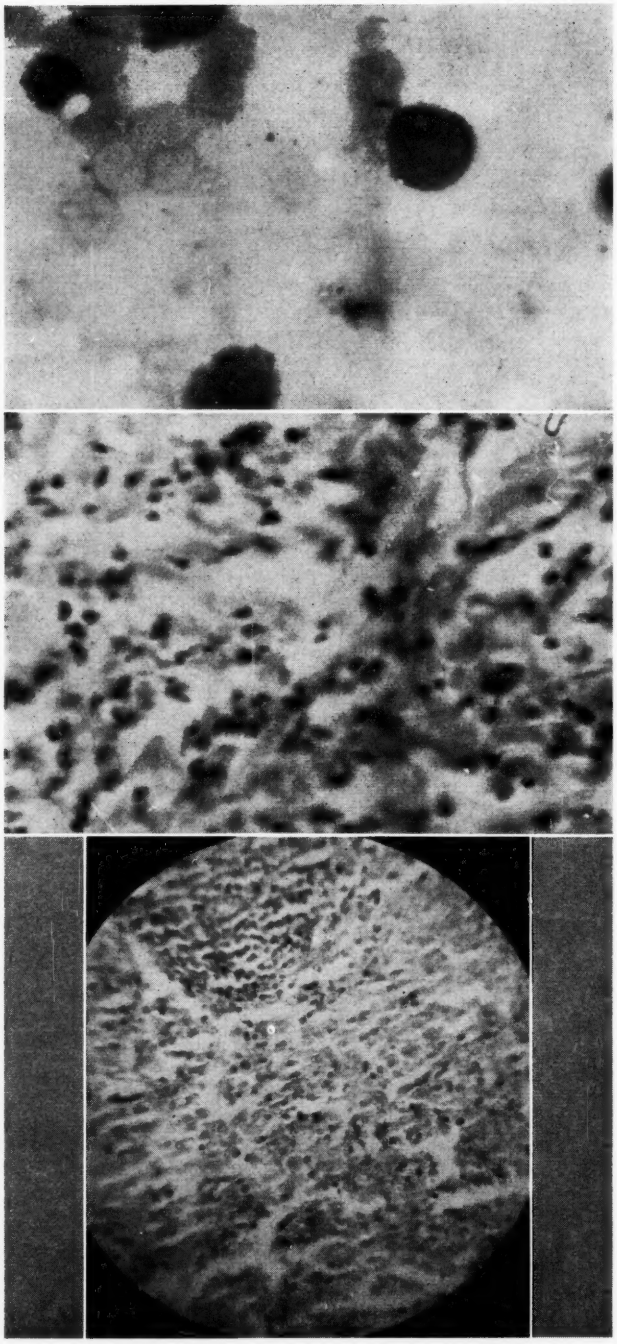


Fig. 4.—Tissue sections from a rabbit that had received injections of crude beef liver extract for a total period of six weeks, with the simultaneous administration of nitrogen mustard during the fifth and sixth weeks. *A*, Femoral bone marrow smear, showing a hypoplastic and agranular marrow with an absence of eosinophilic granular precursor cells; *B*, skin site that had been injected locally with crude beef liver extract forty-eight hours previously, showing an infiltration of eosinophils throughout the corium with associated interstitial edema; *C*, spleen, showing an absence of eosinophilic infiltration, a few iron-containing macrophages, some disruption of the parenchyma, and atrophy of a Malpighian corpuscle in the upper portion of the section.

splenic parenchyma. The lymphoid areas of the white pulp, the Malpighian corpuscles, showed atrophic changes (Fig. 4, *C*).

Bone marrow smears: Microscopic study of the femoral bone marrow of all animals in this group demonstrated an agranular type of marrow with a marked leukopenia. A few scattered young myelocytes, lymphocytes, and an occasional neutrophil were also observed. A marked eosinopenia of the marrow cells was evident (Fig. 4, *A*).

Antibody determinations: Precipitating antibodies to beef liver extract were demonstrated in the sera of only two of the six animals at the end of the six weeks of liver extract injections.

TABLE III. TOTAL CIRCULATING EOSINOPHIL AND WHITE BLOOD CELL COUNTS OF THE SIX RABBITS OF GROUP III, TREATED INITIALLY WITH NITROGEN MUSTARD AND FOLLOWED BY THE SIMULTANEOUS ADMINISTRATION OF NITROGEN MUSTARD AND LIVER EXTRACT

ANIMAL NO.	BEFORE TREATMENT	1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK
	Eosinophils per Cubic Millimeter				
1 C	1,554	555	289	466	222
2 C	821	100	77	255	222
3 C	355	133	255	270	66
4 C	1,465	111	133	111	66
5 C	655	122	127	84	77
6 C	1,100	108	100	244	55
	Mean:				
	992	188	164	238	118
	Leukocytes per Cubic Millimeter ($\times 10^{-3}$)				
1 C	24.5	7.8	9.1	7.5	3.5
2 C	18.7	4.3	2.9	6.4	3.2
3 C	16	3	2.1	6.2	2.3
4 C	22	5.2	4.3	4.8	4.3
5 C	16.6	4	3.8	6.2	2.7
6 C	18.5	7.4	8	7.5	1.9
	Mean:	5.3			
	19.4		5	6.4	3

Each animal received only nitrogen mustard for two weeks, followed by simultaneous administration of nitrogen mustard and beef liver extract for an additional two weeks.

Group III (Receiving Nitrogen Mustard for Four Weeks With the Simultaneous Administration of Beef Liver Extract During the Third and Fourth Weeks.)—

Peripheral blood counts: All six animals of this group developed an eosinopenia and leukopenia during the first two weeks of nitrogen mustard treatment. The total number of both peripheral blood eosinophils and total white blood cells decreased significantly from an average of 992 eosinophils and 19,384 white blood cells per cubic millimeter to 164 eosinophils and 5,034 white blood cells per cubic millimeter, respectively. With the addition of beef liver extract to the nitrogen mustard administered during the third and fourth weeks, there was an initial slight, transient, fluctuating increase followed by a decline in the total numbers of both the circulating eosinophils and the leukocytes (Table III, Figs. 1 and 2).

Skin sections: Microscopic examination of the skin sites locally injected with beef liver extract at the end of the fourth week, just prior to sacrifice,

exhibited a localized infiltration of eosinophilic granular cells within the epidermis and corium of all six animals. An associated interstitial edema was also evident (Fig. 5, *A*).

Spleen: In sections of the spleens studied microscopically after sacrifice, only occasional eosinophilic granulocytes were noted scattered throughout the edematous and disrupted splenic parenchyma. Atrophic changes of the lymphoid areas of white pulp, the Malpighian corpuscles, were evident (Fig. 5, *B*).

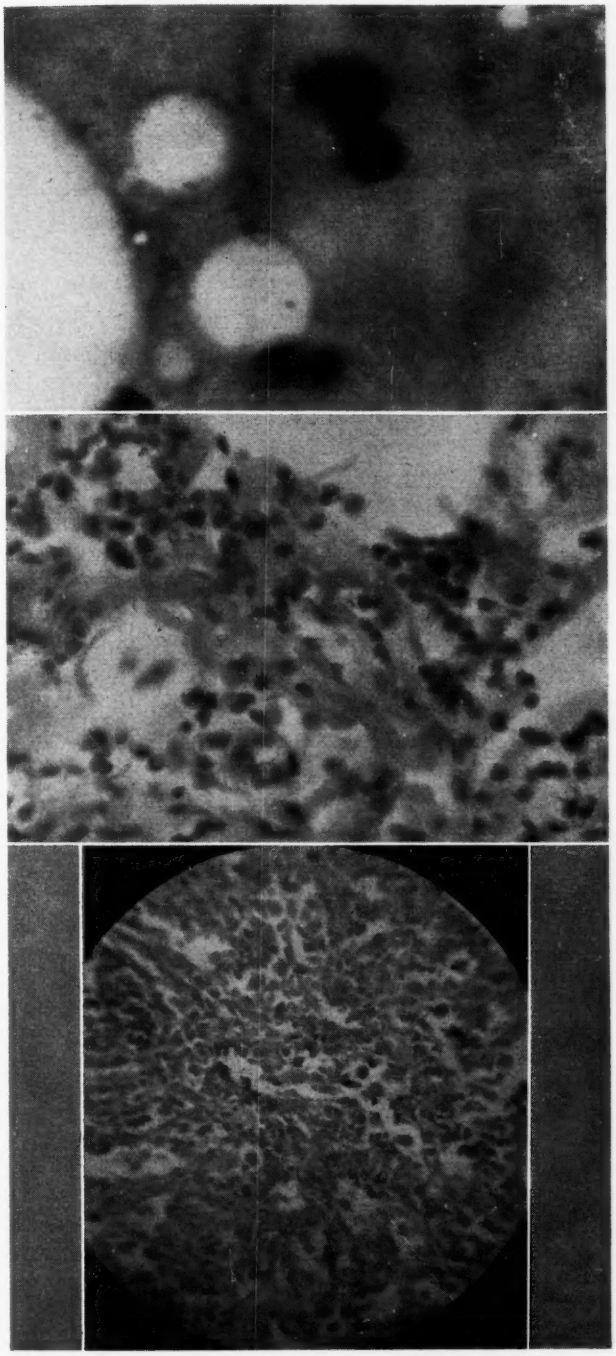
Bone marrow smears: Microscopic study of the femoral bone marrow of all animals in this group demonstrated a hypoplastic agranular type of marrow, with a marked leukopenia and eosinopenia. A few scattered granulocytic leukocytes were present (Fig. 5, *A*).

Antibody determinations: Precipitating antibody could not be demonstrated against beef liver extract in the sera of any of the six animals at the end of the four-week experimental period.

DISCUSSION

Crude beef liver extract was administered to the rabbit by multiple parenteral routes of introduction in an attempt to reach the bone marrow, spleen, and lymphoid tissue and provide these sites with the required stimuli for formation of eosinophil cells and/or antibody production. A maximum circulating blood eosinophilia was thus effected within a period of three weeks of repeated administration of this heterologous species protein. Since cell counts were taken at weekly intervals, it is possible that the exact points of maximum rise and fall in eosinophil levels were not recorded, inasmuch as hourly and daily variations have been pointed out by Speirs, Wenck, and Dreisbach.[23] Although there was an initial associated rise of total circulating leukocytes, the lack of continued parallel fluctuations in both total leukocyte and eosinophil levels resembled a similar pattern noted in sensitized mice by Speirs and associates.[23]

The intradermal injection of crude beef liver extract adsorbed on aluminum hydroxide gel resulted in a localized cutaneous infiltration of eosinophilic granular cells within twenty-four to forty-eight hours after administration. The local accumulation of eosinophils around sites of injected antigen has been described,[1, 14, 19, 20, 43] and Campbell[20] attempted to correlate the insolubility of antigen and the presence of –SH groups with an ability to elicit eosinophilia. Samter[3] has noted the failure of nonspecific protein injection to result in eosinophilia in sensitized guinea pigs. Similar findings had been noted by Campbell[20] in studies with animals of this same species and by Speirs[5] in studies with mice. However, this did not appear to be the case in our study, which employed rabbits. Local tissue eosinophilia occurred within only twenty-four to forty-eight hours after the intradermal administration of crude beef liver extract in all animals, regardless of previous treatment with this material. These microscopic tissue findings also occurred in liver-treated animals independent of the detection of specific circulating antibodies to beef liver. Suppression of the antibody-producing mechanism by the lymphoid



A.

B.

C.

Fig. 5.—Tissue sections from a rabbit that had received injections of nitrogen mustard for a total period of four weeks, with the simultaneous administration of crude beef liver extract during the third and fourth weeks. *A*, Femoral bone marrow smear, showing a hypoplastic marrow with an absence of eosinophilic granular precursor cells; *B*, skin site injected locally with crude beef liver extract forty-eight hours previously, showing an infiltration of eosinophils in the corium with an associated interstitial edema; *C*, spleen, showing a few iron-laden macrophages and disruption of the splenic parenchyma with an absence of eosinophilic cell infiltration.

cytotoxic action of nitrogen mustard apparently was not an interfering factor. The studies by Redd and Vaughn[26] also suggest that active antibody production is not the sole prerequisite for the development of eosinophils. The possibility of acquired antibody of either a fixed tissue or a nonprecipitating serum type incapable of being detected by the techniques employed here cannot be ruled out. However, these findings suggest that the development of localized infiltration of eosinophilic granulocytes within cutaneous sites may have arisen as a tissue response to the presence of foreign protein apart from any active association with mechanisms involving circulating antibody.

In addition to the cutaneous eosinophilic cellular infiltration and the eosinophilia of the peripheral blood and bone marrow, eosinophilic granulocytes were also found throughout the splenic parenchyma of the rabbit following treatment with beef liver extract. Schwartz[13] had similarly reported splenic concentrations of eosinophils following intravenous injection of foreign protein and within other lymphoid organs associated with local infection. This might possibly be explained by the studies of Vaughn,[6] who, in tracing the path of the eosinophil from its site of formation in bone marrow to the blood stream, other organs, and lymphatic system, demonstrated its eventual extraction and destruction by the spleen.

In those rabbits whose only treatment consisted of crude beef liver extract, the marked granular eosinophilia of the bone marrow precursor cells explained the hematopoietic source of increased numbers of mature eosinophilic granular leukocytes found in the peripheral blood and splenic parenchyma. The marrow was completely congested with intact mature and immature eosinophilic granular leukocytes and extracellular, nonphagocytized, red-staining granules. The exact reason for this latter finding is not apparent. It might be that some of the closely packed intact eosinophil cells had been traumatized during technical preparation for study; perhaps a spontaneous rupture of eosinophil cells occurred secondary to the increased production of intracellular granules in response to the foreign protein stimulation on the undifferentiated marrow stem cells or intermediate granulocytic prototypes involved in eosinophilic maturation; or it may even be possible that the accumulation of extracellular granules indicates an exceeding of the granulocyte's ability to phagocytize products resulting from the intense foreign protein or antigenic stimulation. In this connection, Rappaport[37] called attention to the large number of ruptured eosinophils and extracellular eosinophilic granules found in allergic nasal mucosa.

The deleterious effect of nitrogen mustard on peripheral blood polymorphonuclear neutrophils and lymphocytes and their precursors in hematopoietic tissues has been well documented,[44] but very little has been reported regarding the effect of nitrogen mustard on eosinophils. In clinical studies Bateman and associates[45] reported an early reversible decline of marrow eosinophils in patients to whom nitrogen mustard was administered intra-arterially, and Jiménez Díaz and colleagues[46] noted a decrease in circulating eosinophils in

patients with rheumatoid arthritis and asthma who were undergoing experimental treatment with nitrogen mustard. These latter investigators suggested a similarity of action between nitrogen mustard and ACTH, but the report of Cohen and co-workers[47] could not confirm this observation. The studies of Butch and colleagues[48] are of interest in demonstrating the extreme radiosensitivity of the eosinophil, especially in view of the reported similarity of action of nitrogen mustard and roentgen rays in their effect on bone marrow cells.[49] The results of the experiments reported here demonstrate the ability of nitrogen mustard to effect an eosinopenia along with a predicted leukopenia and lymphocytopenia, to reverse an induced peripheral blood eosinophilia to eosinopenic levels, and to prevent an elevation of circulating eosinophils despite the presence of an eosinotactic stimulus. The hypoplastic and aplastic bone marrow with the lack of eosinophilic granular cell precursors explains the manifestation of circulating eosinopenia in animals treated with nitrogen mustard. The initial effect of the nitrogen mustard apparently resulted in a suppression and/or destruction of eosinopoietic as well as leukocytic stem cells which were unable to recover sufficiently to respond to foreign protein stimulation. The splenic parenchyma also exhibited an eosinopenic effect along with the characteristic atrophy of lymphoid tissue resulting from nitrogen mustard action.

The development of a peripheral blood eosinopenia from either pre-existing, normal, or eosinophilic levels did not limit the development of tissue infiltration with eosinophilic granular cells resulting from localized intradermal stimulation. Although not definitely known, it is possible that eosinophils may originate in tissue sites other than bone marrow and respond by migrating to the skin site of foreign protein localization. This opinion is at variance, however, with the opinions of other investigators, such as Samter[3, 4] who feels that bone marrow is the sole site of eosinopoiesis and that any increase in eosinophils is due to their release from the bone marrow with transportation through the vascular bed and subsequent migration into the shock tissue following nonfatal anaphylaxis. Any alternate explanation would assume a tissue origin of eosinophils resulting from such an anaphylactic reaction with a subsequent increase of blood eosinophils due to an overflow into the general circulation. The approach of Samter and his co-workers[33] to this problem also included an attempt to free the peripheral vascular bed of eosinophils prior to anaphylactic shock by employing the lytic effect of ACTH on circulating eosinophils. However, their results were inconclusive in determining whether eosinophils originated in or migrated out of the peribronchial shock tissues of the guinea pig. It was pointed out by these authors that circulating eosinophils may not be lysed or otherwise destroyed but that perhaps they may be driven into the tissue by the administration of ACTH. In view of the known destructive action on mature and immature leukocytes and the demonstrated bone marrow eosinopenic effects of nitrogen mustard, a similar "driving effect" cannot be attributed to this chemical agent in explaining localized cutaneous accumulations of eosinophils.

Samter's[3] report on studies with sensitized guinea pigs indicated a definite correlation between the presence of large numbers of eosinophils in the blood and their presence in the lungs. However, Jadassohn's[50] experiments demonstrated that human skin is capable of developing a local eosinophilia after such traumatic procedures as mechanical irritation and the local injection of morphine, atropine, or pilocarpine—even in the absence of blood eosinophilia. In a clinical study of various skin diseases, Burkhart and Montgomery[51] were unable to find any correlation between the eosinophils of tissue and blood. Despite the availability of a great deal of carefully documented evidence, the relationship of blood eosinophilia to that occurring within tissue sites still remains a poorly understood biologic phenomenon. Perhaps these discrepancies can be explained by true species differences, or even by the existence of multiple etiological mechanisms responsible for eosinopoiesis. It is admittedly possible that, even in the presence of a peripheral blood eosinopenia in rabbits treated with nitrogen mustard, the few remaining circulating eosinophils were sufficient in numbers to meet the demands of the tissue stimulus afforded by the local introduction of foreign protein. However, the facts that (1) a significant eosinophilic cellular infiltration of skin developed in the presence of peripheral blood eosinopenia, neutropenia, and lymphocytopenia and (2) eosinotactic mechanisms in localized cutaneous tissue sites were effective in animals whose hypoplastic marrow would not permit an eosinophilic response to the same foreign protein stimulus suggest that eosinophilic granulocytes of skin may have a tissue origin apart from peripheral blood and bone marrow hematopoiesis. Possible alternate sites of such formation, however, are at present a matter only for speculation and for further investigation.

SUMMARY

1. A total of eighteen rabbits were given injections of crude beef liver extract by intramuscular, intraperitoneal, and intravenous routes in an attempt to induce an eosinophil cell response in peripheral blood, bone marrow, and splenic parenchyma.

2. Six of the animals received only crude beef liver extract for a six-week period. These developed a maximum rise in circulating blood eosinophils by the third week.

3. Six of the rabbits also received beef liver extract for a six-week period, but when a significant rise of circulating eosinophils had been reached by the third to fourth week, nitrogen mustard was administered intravenously and simultaneously with the liver extract during the remainder of the experimental period. The previously noted eosinophilia was then reversed to eosinopenic levels and was associated with leukopenia and lymphocytopenia.

4. Six of the animals received nitrogen mustard for two weeks prior to the injection of beef liver extract. This resulted in the development of peripheral blood eosinopenia and leukopenia. When eosinopenia had been established, the simultaneous administration of beef liver extract with nitrogen mustard failed to raise significantly the level of circulating eosinophils.

5. Crude beef liver extract adsorbed on aluminum hydroxide gel was injected intradermally into each animal at the start of the experiment, at the height of circulating eosinophilia, prior to the initial administration of nitrogen mustard, and at the conclusion of the course of parenteral beef liver extract injections. All locally injected skin sites were biopsied forty-eight hours following intradermal injections.

6. Infiltrations with eosinophilic granular cells were noted in the epidermis and corium of all animals, regardless of whether peripheral blood eosinophilia was present, had been reversed, or had been prevented from developing by the administration of nitrogen mustard.

7. Eosinophilic cellular infiltration into the splenic parenchyma was demonstrated microscopically in all rabbits to whom only beef liver extract was administered. In those who had also received nitrogen mustard, an absence of eosinophilic infiltration and the presence of atrophy of the lymphoid follicles were observed.

8. All animals treated with only beef liver extract exhibited marked hyperplastic granular eosinophilic bone marrows. Those who had been treated with nitrogen mustard prior to or simultaneously with liver extract exhibited agranular bone marrows with absence of eosinophilic precursor cells.

9. Precipitating antibodies to beef liver extract could not be demonstrated in the sera of all animals treated with this material. The development of cutaneous eosinophilic cellular infiltration could not be correlated with associated antibody response or antigen-antibody reaction.

10. Within the limitations of this experimental procedure, these results suggest that in the rabbit the development of a tissue eosinophilic cellular response may not be related to the presence of circulating peripheral blood eosinophils and local tissue eosinophils of the skin may have a source of origin independent of peripheral blood eosinophils. However, the exact origin or mode of their formation is not apparent.

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HYDROXYZINE (ATARAX) IN CHRONIC URTICARIA AND IN ALLERGIC MANIFESTATIONS

Clinical Observations in Man and Experimental Studies on Asthma in Guinea Pigs Produced by Several Agents

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TRANQUILIZING drugs in general have an equivocal effect on the manifestations of allergic disease. Quite accidentally we found ourselves launched on a series of observations which indicate that hydroxyzine (Atarax) may be one exception to the rule, particularly in the relief of chronic urticaria. Our first experience in this connection dealt with the following case.

Mrs. F. W., who was first seen by us in September, 1956, had a history of generalized urticaria which had been occurring daily for an entire year. No aggravating factors could be elicited. The patient has a daughter who is allergic. Except for some delayed reactions, skin tests for foods were negative. Elimination of these foods had no effect on the symptoms, and further trial diets did not help. Several potent antihistamines were tried, with no significant results. The patient's husband, a physician, tried various medications in the course of her illness, both before and after she consulted us. He also found that the antihistamines were not effective. In desperation he began to use other types of medications, including sedatives and tranquilizers. Potent sedatives, meprobamate, chlorpromazine, and various other medications gave no help, but Atarax proved to be very effective. This drug was given originally in 10 mg. doses two or three times a day, and later the dosage was reduced to 10 mg. once a day. Under our observation, Atarax produced complete freedom from urticarial swellings and itching. The patient was seen again one year later. Her urticaria was still present without medication but was completely controlled by Atarax. By varying the time intervals, it was determined that a 10 mg. dose would last for approximately thirty-six hours.

Several aspects of this case interested us greatly when we first noted the results obtained with Atarax. It appeared paradoxical for such a relatively mild tranquilizer to have so potent and prolonged an effect, when sedatives, other more potent tranquilizers, and antihistamines gave no relief. Another argument against the tranquilizing effect of Atarax as an explanation of the

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relief was the fact that the sedative action of the drug is of much shorter duration than the antiurticarial action. As a result of this observation, other similar patients were placed on this drug, with excellent results. It seemed appropriate, then, to subject Atarax to further clinical testing and to laboratory investigation.

CLINICAL STUDIES

Atarax was obtained in 10 and 25 mg. plain uncolored capsules. We observed its effects on a variety of allergic diseases as they were encountered both in private practice and in the clinic. We attempted to determine the effectiveness of this drug and to learn whether the benefit derived from it might be ascribed to its tranquilizing or its antiallergic properties. Usually we began with a dose of 10 mg. three times a day. In the absence of appreciable relief, this was often increased to 25 mg. three or four times daily. If results were obtained with 10 mg. doses, we tried to reduce this to determine the minimum dose necessary for relief. Sixty patients are included in this study. As some had multiple symptoms, the totals of the various manifestations add up to more than the total number of patients.

The most striking results were seen in those patients with chronic urticaria of undetermined etiology. Fifteen of seventeen patients obtained complete relief, and two had fair to good relief. There were no complete failures. In several patients, however, relief lasted for only a few weeks, at which time the drug appeared to lose its effectiveness. A number of the patients who maintained their relief were able to stay on 10 mg. doses as infrequently as every twenty-four to seventy-two hours. In some of the patient with chronic urticaria, Pyribenzamine gave good relief from the itching but not so much from the swellings; however, the effect of each dose lasted only four to six hours. In contrast with chronic urticaria patients, only one out of four patients with acute urticaria had significant relief. One of the patients who failed to obtain relief had a "serum sickness" type of urticaria from penicillin.

Results in atopic dermatitis were judged primarily on the basis of relief of the itching. One patient had excellent relief, four had fair to good relief, and in two cases no effect was noted. Although such results were not completely satisfactory, poorer results were obtained with other tranquilizers. Thus, it is possible that Atarax has some antiallergic effect in this type of case also. In two cases of contact dermatitis, one patient had some relief and the other had none. Six patients with seasonal hay fever had no relief, and three experienced fair results. Slightly better results were obtained in patients with vasomotor rhinitis. Six patients had fair to good relief, and four had no benefit. Of thirteen patients with asthma, seven had fair to good relief and no effect was noted in six. Better results were obtained in patients with dermographism: one had excellent relief and two had good relief. One patient with a physical hypersensitivity to cold, who had had only slight relief with antihistamines, obtained excellent results with Atarax.

Side effects were noted in eleven patients. One claimed stimulation from Atarax, while the other ten noted mild sedation. In no case in which good therapeutic results were obtained was it necessary to discontinue this drug because of side effects.

EXPERIMENTAL OBSERVATIONS

In these studies anaphylaxis was produced by passive intravenous sensitization of the guinea pig, using guinea pig antiovalbumin serum twenty-four hours prior to challenge with an aerosol of egg albumin. Animals were also subjected to aerosols of histamine, serotonin, or acetylcholine. With each agent, the concentration of the solution for the aerosol was that strength which by previous assay was found to produce asthma in guinea pigs in less than two minutes. Several drugs—Atarax, Sparine, Thorazine, Compazine, and Suavitil—were evaluated by intraperitoneal injection into guinea pigs prior to the appropriate aerosol challenge. A total of 129 guinea pigs were subjected to the various challenges after varying intervals following pretreatment with different dosages of the drugs. Two antihistaminic drugs—Pyribenzamine and Thenylene—were included also for comparison. Table I gives a short summary of the data obtained.

TABLE I. EFFECT OF SOME DRUGS ON ASTHMA PRODUCED BY VARIOUS AGENTS

DRUG	DOSE (MG./KG.)	AVERAGE TIME IN SECONDS* EXPOSED TO AEROSOL BEFORE ASTHMA OCCURRED				
		SEDATION	OVALBUMIN IN SENSITIZED ANIMALS	HISTAMINE	SEROTONIN	ACETYLCHOLINE
Atarax	10	No	600	537	556	396
Sparine	20	Yes	402	238	–	–
Thorazine	10	Yes	280	175	–	–
Compazine	10	No	128	98	–	–
Suavitil	2	No	151	117	–	600
Pyribenzamine	2	No	567	586	402	399
Thenylene	6	No	600	600	116	–
Control	0	No	115	65	101	68

*The maximum time observed is 600 seconds. Animals remaining for 600 seconds will probably tolerate the aerosol indefinitely, since a number of such animals were followed for twenty minutes without further symptoms.

Atarax, in a dose of 10 mg. per kilogram, was completely effective against anaphylaxis and nearly so against histamine. Protection against anaphylaxis or histamine is manifested within one-half hour, remains at a maximal level for six hours, and diminishes to 50 to 75 per cent of this value within eighteen hours, but it still exerts definite activity after twenty-four hours. With Pyribenzamine the major part of these effects had disappeared by the end of four hours. Atarax was also quite effective against aerosols of serotonin and acetylcholine. At this dosage of 10 mg. per kilogram, no sedative effects were observed. With the other drugs, much less activity was found against anaphylaxis or against histamine, even at dosages large enough that sedation was noted.

DISCUSSION

From the foregoing clinical and experimental findings, it is quite apparent that the sedative action of Atarax is not responsible for the results in animals or allergic man. In the anaphylactic challenge in the guinea pig every drug tried, including Pyribenzamine and Thenylene, showed an efficacy comparable to its antihistaminic effect. It is also apparent that a good antiacetylcholine effect, such as that obtained with Suavitil, gave virtually no protection against anaphylaxis. Although the antiserotonin effect roughly paralleled the antianaphylactic effects of Atarax and Pyribenzamine, Thenylene had no antiserotonin action even though its antianaphylactic activity was excellent.

The effectiveness of Atarax in most allergies was not striking. It should be noted, however, that the dose of the drug used in man (10 to 25 mg.) is approximately one-fifth to one-half of the average Pyribenzamine dose. In the animal experiments, on the other hand, the effective dose of Atarax was five times that of Pyribenzamine. The excellent results obtained with Atarax in chronic urticaria in doses of one-fifth or less of those used for Pyribenzamine cannot be explained by its antihistaminic, antiserotonin, or antiacetylcholine action, or by a combination of these actions. These findings suggest that there is an additional mediator in chronic urticaria and that the mechanism involved is not the same as in experimental anaphylaxis or in the manifestations of recognized allergic origin. As a corollary to that, it may be significant that in the majority of cases of chronic urticaria no obvious allergic causes are discernible.

SUMMARY

1. Atarax gives relief in the majority of cases of chronic urticaria and dermographia.

2. Atarax is less effective in other allergic diseases.

3. Indirect evidence points to the possibility that there is a mediating mechanism in chronic urticaria besides the possible ones of histamine, acetylcholine, or serotonin.

4. Experimental studies in the guinea pig indicate that Atarax is a long-acting, potent, antianaphylactic, antihistaminic, antiserotonin, and antiacetylcholine agent. For the most part, other tranquilizing or antihistaminic drugs have only part or none of these effects.

5. The antihistaminic activity of these drugs appears to be the most important feature determining its antianaphylactic action in guinea pigs.

ETIOLOGICAL AGENTS OF RESPIRATORY ALLERGY IN TROPICAL COUNTRIES OF CENTRAL AND SOUTH AMERICA

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CONTRARY to what has happened in the United States, where Durham,[1-4] Shapiro and Rooks,[5] Spain,[6] and many others[7-9] have extensively studied the incidence of air-borne pollens and its relation to hay fever, in the countries of the tropical zone a comparative study of the incidence of allergens in the air has not been carried out as yet. It is only in the last few years that investigations on atmospheric pollens, fungi, and other etiological agents of the respiratory allergies in Central and South America have begun.

These studies now make possible a conjoint review of the allergenic flora of the different countries in the tropical zone, from Mexico in the north, with Central America and the Caribbean islands, to Peru, Bolivia, and Brazil in the south.

The present study deals with the distribution and incidence of air-borne pollens and fungi and also presents some new figures on the frequency of asthma, allergic rhinitis, and "skin allergy" in the hospital population. The study is based partly upon data published by previous workers and chiefly upon counts of pollens and molds made, under my direction, in the air over certain areas in South America and upon the results of skin tests made with extracts of these pollens and molds on my patients.

AIR-BORNE POLLENS AND FUNGI

The Most Important Plants and Pollens.—In the countries of the temperate zone, with the four seasons in the year, the time of pollination of each botanical species is clearly defined and the postulates of Thommen[10] are fulfilled, but in the countries of the tropical zone there are only two seasons, which in some regions are hardly distinguishable—a rainy one and a dry one. They occur at different times in the various geographical areas. In places where the flowering of plants depends exclusively on the rains, the pollination periods of the different species are quite well differentiated. The pollen concentration is never as high, however, as it is in the countries of the temperate zone. In the places where there is soil irrigation and the temperature is appropriate, the weeds, grasses, and shrubs flower and pollinate during the whole year.

In countries of low altitude the atmospheric humidity is quite high, but in the mountain regions the humidity is lower. In both, however, flowering and pollination occur mostly with rains.

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For regions which are located between sea level and 1,000 feet (except the coasts that are influenced by the Humbolt stream), the median annual temperature is approximately 76° F., with a maximum of 82° F. The median atmospherical humidity is 92 per cent to 95 per cent. The average annual rainfall reaches 2,000 to 3,000 mm. The temperature decreases with the increasing altitude, approximately 1° F. for each 500 feet. The rain and atmospheric humidity decreases also, but there are large differences between one place and another.

In the countries of the temperate zone of both North[1, 2, 6] and South[11-13] America, the plants of the *Compositae* family, of which the genus *Ambrosia* (ragweed) is the most important, produce a high number of allergic reactions. The genus *Artemisia* (wormwood) and the genus *Xanthium* (cocklebur) are less important than *Ambrosia*. These composite plants are followed by plants of the *Chenopodiaceae* (goosefoot, pigweed) family, which embraces several genera, the different species of which may be dominant in different localities. Thus, *Chenopodium album* (lambs'-quarters), *Salsola tenuifolia* (Russian thistle), *Atriplex* (Orach), and *Kockia* (Roth) all belong to the *Chenopodiaceae* family, and in North America one or another may be important locally.

Similarly, the *Amaranthaceae* family includes several genera, of which *Amaranthus* (careless weed) and *Acnida* (water hemp) are noted most often.*

Grasses and certain trees which produce pollen in abundance at an earlier season than the weeds cause almost as much trouble in the form of allergic reactions in North America as do the weeds.

In the countries of the equatorial zone most of these plants are scarce. Table I shows the species important in distribution or in allergenic power that grow in the equatorial zone.

In the middle and north of Mexico during the high pollination days, 100 or more pollen grains can be found per square centimeter per day. Descending toward the equatorial line, however, the number of pollens considerably diminishes. In Quito, for example, it rarely reaches 10 or more. It is interesting to notice that, descending from the equator toward the south, the amount of pollen grains increases again. In Brazil,[32, 35] between Rio de Janeiro and São Paulo, 50 to 60 and even 100 pollen grains were found, according to the place and the days of highest pollination. The low concentration of pollens in the air is not due entirely to the scarceness of plants. Almost the entire tropical zone is very rich in grasses, but, nevertheless, there are very few grass pollens in the air. Temperature and humidity play an important role.

In the low regions, as mentioned before, the atmospheric humidity is such that it probably interferes with diffusion of pollens, and in the mountainous regions over 12,000 feet where the grasses are abundant, probably because of the cold, the plants flower very little.

*The classification of families and genera is taken from Gray's *New Manual of Botany*, ed. 7, New York, Cincinnati and Chicago, 1908, American Book Company.

TABLE I. FAMILIES AND GENERA OF THE MAIN ALLERGENIC PLANTS OF THE COUNTRIES OF THE TROPICAL ZONE

PLANT	MEXICO[14-19]	CENTRAL AMERICA[20]	CUBA, PUERTO RICO[21-23]	COLOMBIA[24, 29]	ECUADOR[25-29]	PERU[30]	BOLIVIA[31]	BRAZIL[32-37]	VENEZUELA[29]
Gymnosperms									
Pinaceae									
Pinus	+++		+	+	+	+			
Cupressus	+++			+	+	+		++	
Angiosperms									
DICOTYLEDONES									
Fagaceae									
Quercus	+++								
Polygonaceae									
Rumex	+	+	+	+	+	+	+	+	+
Chenopodiaceae									
Chenopodium	+++	+	+	++	+++	++	++	++	++
Salsola	+								
Kochia	+								
Atriplex								+	
Amaranthaceae									
Amaranthus	+++	++	+	+++	+++	++	+	+	++
Platanaceae									
Platanus	++			+	+			+	
Leguminosae									
Acacia					+	+			
Mimosa				+	+	+			
Anacardiaceae									
Mangifera	+	++	++	++	++			++	
Plantaginaceae									
Plantago	+		+	+	+	+		+	+

| | | |

Trees are important in Mexico and to a lesser extent in the south of Brazil. Weeds, such as *Ambrosia,* although abundant in Cuba and Brazil, are of secondary importance. In the other countries *Ambrosia* is rarely found.

Plants of the *Amaranthaceae* and *Chenopodiaceae* families are certainly the most important species in many tropical countries, followed by the *Compositae* family, of which *Helianthus* is of interest in Mexico, *Viguieria* in Cuba, and *Franseria* in Ecuador. Grasses are even less important than weeds, but *Melinis* would be of interest in Brazil and *Cynodon, Poa, Holcus,* and *Lolium* in the other countries.

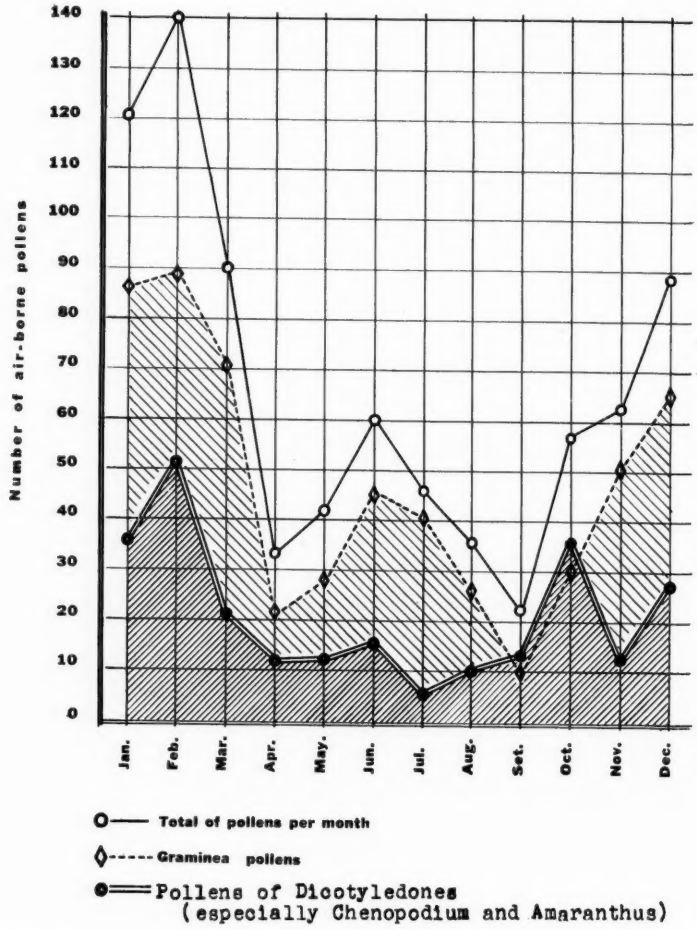

Fig. 1.—Monthly frequency of pollens in the atmosphere of Quito. In the ordinate are the number of pollens per month and per square centimeter.

Pollination Calendar.—The pollination calendar varies with the rains. From one to two months after the rainy season has started, the number of pollens in the air gradually begins to increase.[20, 21, 25] If it stops raining for a few days or weeks and the sun shines and the winds blow, the concentration

of pollens in the air can reach its maximum; inversely, when there are rainy days with clouded sky and plenty of atmospheric humidity, the air is cleansed of pollens.

In Cuba the rainy season is from May to November, on the coast of Ecuador it is from December to April, and in other places the rain starts in October and continues until May or June.

I have made special studies of two places: My own city, Quito, which is the capital of Ecuador, is in a high mountainous region 9,242 feet above sea level and 10 miles south from the equator. The other one is Cali, in Colombia, about 350 miles north of Quito. Cali lies in a subtropical valley at an altitude of only 3,000 feet between the Occidental and the Central Cordillera mountain ranges. Comparative studies of these two places are interesting.

Both Quito and Cali have rains from October to May, but in Cali the rains are more abundant. The month with the greatest precipitation varies from one year to another. It may be October, but it is more frequently April.

Counts of pollens were carried out in accordance with the method described by Durham,[3] using the standard air-sampling device, recommended as "standard" by The American Academy of Allergy,[38] which permits the calculation of the concentration of pollens per cubic yard of air.[39]

Data from Quito are based upon daily counts during 1948 and 1949 and those from Cali are based upon counts during the second part of 1954 and the first part of 1955. The pollination calendar of Quito[29] is shown in Fig. 1. When the rains begin in September, pollens, especially those from grasses, increase to reach a maximum in January, when the number of rainy days decreases. Beginning in March, the number of pollens falls off until the end of April and then it increases slightly again until June. In July the number of pollens decreases, and during August and the first part of September it remains at a low level. The pollination calendar for Cali is similar, but the number of pollens per square centimeter is three to five times greater than that from Quito. There are some differences with regard to the prevailing botanical species. In Cali there are more tree pollens (*Leguminosae, Bignoniaceae,* etc.). In Quito the pollens of *Amaranthus quitensis* predominate, and in Cali those of *A. spinosus* are numerous. Cali has an appreciable number of grass pollens.

Mold Spores.—Mold spores are especially important in the tropics. As demonstrated by Cadrecha-Alvarez[46] and others,[47, 48] the climatic conditions, being very favorable to the growth of fungi, may result in a high concentration of spores in the air which, in turn, cause an increased incidence of allergic diseases.[42]

In an attempt to study the air-borne fungi quantitatively in comparison with pollens, the spores were counted in Quito and Cali by the same method as used to count the pollens. (For their identification, the method described by Harris[40] was used, but the Petri dishes were left within the standard air-sampling device only twelve minutes.) In Quito,[29] the annual curve of incidence of fungus spores in the air was similar to that of pollens. The highest

TABLE II. THE MOST IMPORTANT AIR-BORNE FUNGI IN SOME TROPICAL COUNTRIES

(ARRANGED ALPHABETICALLY)

FUNGI	MEXICO		CUBA		COLOMBIA	ECUADOR	PERU	BRAZIL	
	MEXICO CITY[41, 42]	WESTERN MEXICO[43]	HAVANA[44-46]	CUBA[21]	CALI	QUITO[29]	LIMA[47]	RIO DE JANEIRO[48, 49]	SÃO PAULO[50]
Alternaria	+++	+++	++	+++	+++	+	++	+++	
Acrothecium		+		+			+		
Aspergillus	++	++	++++	+++	++++	+++	++++	++++	++
Candida	+				+		+	++	++
Cephalosporium	+							+	
Chaetomium		+		++					
Cheothonium		+							
Fusarium	++		+	+		+	++		
Helminthosporium		++		+	+		++	+	
Hormodendron	++++	++++	+++	+++	++++	+	+++	++++	++++
Monilia	+			+		+	+	+	
Mucor	++	+		+	++	+++	++	++	++
Penicillium	++++	+++	++++	+++	++++	+++	++++	++++	++++
Phoma		+		+			+		
Puccinia					+	+			
Pullularia									+
Rhizopus	+	+		+	+++	+++	+++	+	+
Rhodotorula					+	+		++	+++
Torulopsis							+	+	++

Number of plus signs (+) refers to the relative abundance of air-borne spores.

incidence occurred in January and the least in August. The amount of spores that settled on a square centimeter was at least ten times larger than the total amount of pollens. This ratio prevailed throughout the whole year. Since the "conversion factor"[39] is relatively great for spores, if the concentration per cubic yard is considered, then the concentration of spores was actually 10 to 100 times higher than that of pollens. Cali, at low altitude and with more atmospheric humidity, had even more molds than Quito.

In other places where quantitative studies were carried out, as in Cuba[44, 45] and Brazil,[48] a relative abundance of air-borne fungi has been found. Unfortunately, different methods were used by the various authors, and a direct comparison of their data is meaningless. However, for the purpose of summarizing in a single table major findings of these workers, Table II was elaborated under a pattern similar to Table I, but the plus signs used in Table II have a more relative value, rather than an arbitrary one.

Among the most important fungi, as determined by relative abundance or allergenic power, in all the countries studied are *Penicillium* and *Aspergillus*. *Hormodendron* is found very frequently in low and humid places (Havana, Rio de Janeiro, etc.), while in high places (Quito), *Rhizopus* and *Mucor* are much more abundant than *Hormodendron*.

ETIOLOGY AND FREQUENCY

Hay Fever.—In 1946, Oliveiro-Lima and associates[35] asserted that no hay fever exists in Brazil, and the existence or nonexistence of this allergic condition was argued in other countries of the tropical zone. The use of the word *pollinosis* brought a still greater confusion on this particular problem. If we understand *pollinosis* to mean any allergic condition produced by pollens, then pollinosis exists. But hay fever, with that dramatic and intense symptomatology in well-defined periodic form and with epidemic character, practically does not exist in the tropical zone.

Rhinitis due only to pollen is very rare[21, 24, 29, 31, 51, 52] and shows up in a mild form and without strict seasonal character, probably because of the low concentration of pollens in the air and because of the previously mentioned fact that where adequate soil humidity is available the plants pollinate indiscriminately during the whole year.

Asthma and Allergic Rhinitis.—The frequency of asthma and allergic or vasomotor rhinitis varies from one place to another. In a survey among medical students, for example, it was found that in Cali 4 per cent of the students were actually asthmatic, while in Quito there were no asthmatic students. Table III provides other comparative data from the two cities. In Quito the cases of asthma are mild; status asthmaticus is very rare. Skin allergies, however, are relatively frequent. In Cali, on the other hand, the respiratory allergies, and particularly asthma, have a rather high incidence. Of the asthmatic patients in Quito more than two-thirds had the initial onset of asthma while living in an asthmogenic region such as the coast.

It is well known by the physicians practicing in Ecuador that allergic rhinitis and asthma are uncommon diseases in the mountains, whereas in the coastal and southern parts of the country asthma is frequent.

TABLE III. RELATIVE FREQUENCY OF THE ALLERGIC DISEASES

DISEASE	QUITO (ECUADOR) (PER CENT)	CALI (COLOMBIA) (PER CENT)
Asthma	18*	51†
Allergic rhinitis	14	18
Skin allergy (urticaria, eczema, prurigo, etc.)	55	18
Others	13	11

*From a statistical sample of 200 allergic patients.
†From a statistical sample of 100 allergic patients.

In order to present some figures, statistical data from four hospitals—two from Ecuador and two from Colombia, in each country one in a high and the other in a low altitude—were reviewed. The four institutions are general hospitals, each being the largest one in its respective city. In these countries it is customary for those who can pay to go to private clinics. The choice depends on the specialists affiliated with each clinic. The poor people, who cannot pay for private medical assistance, go to these hospitals. In each hospital can be found a cross section of all the pathology in the area with no important discrepancies between the actual incidence of illness inside and outside the hospital; therefore, it is reasonable to assume that data from the individual hospitals can be validly compared.

TABLE IV. RELATIVE FREQUENCY OF HOSPITALIZED ASTHMATIC PATIENTS

PLACE AND HOSPITAL	AVERAGE TOTAL PATIENTS PER YEAR*	PER CENT ASTHMATIC PATIENTS
Bogotá (8,573 feet)†		
San Juan de Dios Hospital	11,000	6.3
Cali (3,000 feet)		
San Juan de Dios Hospital	5,500	6.7
Guayaquil (19 feet)		
Luis Vernaza Hospital	20,000	6.5
Quito (9,242 feet)		
Eugenio Espejo Hospital	6,000	1.85

*Data from the years 1955 and 1956.
†Altitude over sea level.

Table IV presents the relative frequency of asthma in the four hospitals, and the striking difference between the hospital in Quito and the others could be taken as an indication of the different incidence of asthma in the various localities. Altitude does not seem to be the determining factor in such difference. Quito and Bogotá are in the mountains and there is not a great difference in altitude; however, there is much less asthma in the first city than in the second. Evidently other conditions besides altitude determine the frequency of this allergic disease.

The etiology of allergic asthma and rhinitis was studied in patients of Quito and Cali. Most of the patients were referred by internists for their

treatment. Each had a careful history and complete physical examination. Intradermal tests were carried out in each patient. In the preparation of Table V, only those patients in whom the intradermal tests coincided with the findings of clinical history were considered. In some patients clinical exposure by intranasal application of raw materials or extracts was made. The favorable response to the specific treatment with the respective antigens was taken as a confirmation of the intradermal findings.

TABLE V. RELATIVE FREQUENCY OF THE ETIOLOGICAL AGENTS OF THE RESPIRATORY ALLERGIES

ETIOLOGICAL AGENT	ASTHMA		RHINITIS	
	QUITO (PER CENT)	CALI (PER CENT)	QUITO (PER CENT)	CALI (PER CENT)
1. House dust + fungi	16	22	12	35
2. House dust + pollens	7	6	10	15
3. House dust + inhalants	19	16	16	5
4. House dust + fungi + inhalants	38	33	39	25
5. Other combinations	14	13	15	10
6. Only house dust	6	10	5	10
7. Only fungi	–	–	3	–
8. Only pollens	–	–	–	–

The most important etiological agent was house dust; in some cases it was the only etiological agent. In a relatively high percentage of patients, there were two or more etiological agents, such as house dust, fungus spores, and one or more "inhalants" (kapok, sheep wool, cotton, epidermals, etc.). The most frequent combinations of etiological agents were about the same in Quito as in Cali. Pollens had a secondary role. In the statistical sample studied, there were no cases of asthma or rhinitis produced exclusively by pollens.

Although the grass pollens in the air are more abundant than those of other plants, the allergies in which these pollens are involved are uncommonly found. In Quito the pollens of chenopod plants have more importance, followed by those of amaranth plants, while in Cali the pollens of the amaranth plants are the most important.

Allergic Rhinitis and Deflection of the Nasal Septum.—The existence of a deviated nasal septum brought the suspicion that this may cause the rhinitis, but since surgical treatment did not produce any improvement, the deviation of the nasal septum was regarded as only a casual finding. Allergic causes must be considered carefully before surgery is advised.

SUMMARY

In the tropical countries of Central and South America there grow only a few species of plants that produce allergenic pollen, and these are not widely distributed, with the exception of the grasses which otherwise have a feeble sensitizing power. The output of pollens from grasses is very limited in the high mountains because of the lack of flowering of such plants. The most important species correspond to the families *Amaranthaceae, Chenopodiaceae,* and *Compositae.*

The atmospheric conditions, especially the atmospheric humidity and the hard rains, prevent the dissemination of pollen grains in the air. Pollen concentration increases after the rainy season starts. In some regions it reaches the highest level between November and February. The lowest concentration is found during the dry season.

Fungus spores are very abundant in the air. They are found from 10 to 100 times more frequently than the pollens. The spores of *Penicillium* and *Aspergillus* are present in great quantity in almost all the countries of the tropical zone. In the mountains, molds of genera *Mucor* and *Rhizopus* are also important, while in the lowlands *Hormodendron* is dominant. *Fusarium* and *Rhodotorula* are also of some interest.

In the countries of the tropical zone, hay fever practically does not exist, a fact due, at least in part, to the low concentration of pollens in the air. However, cases of nonseasonal vasomotor rhinitis, and especially asthma, are common.

The frequency of asthma varies from one region to another. There are asthmogenic regions, such as the coast of Ecuador and the greater part of Colombia. In these regions incidence of asthma is relatively high. In others, especially that of Quito, asthma is infrequent.

The most important etiological agent of asthma and allergic rhinitis is house dust. Alone or in association with other allergenic substances, this is the etiological agent of almost all the cases. The fungus spores and the group of "inhalants" (kapok, sheep wool, epidermals, etc.) are next in importance. The pollen grains are of secondary importance.

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Editorial

OBSTRUCTIVE PULMONARY EMPHYSEMA

A THREE-DAY conference* covering most aspects of pulmonary emphysema will have been held, by the time this appears, in Aspen, Colorado. The large amount of research devoted to emphysema, culminating in a conference devoted exclusively to this disease, is eloquent testimony for the importance that a nontuberculous chronic pulmonary disease is now accorded. The conference should be of interest to the allergist, not because pulmonary emphysema need fall within his province but because the distinction between the disease and bronchial asthma is of both theoretical and practical importance and at times is far from easy to make. Classifications of asthma, of which there are a number, make room for a form variously referred to as "intrinsic," "idiopathic," or "nonallergic," and a second category called "infectious" is also often included. Among those patients so classified are many who do not meet the criteria for atopy. On the other hand, they "wheeze," and this physical sign tends to be equated with asthma—a deplorable tendency which dignifies a simple and inconstant physical finding with the accoutrements of a complex and as yet poorly understood pathologic process and robs the word *asthma* of useful meaning. If we define asthma as an allergic bronchitis and bronchiolitis, how many of these patients have asthma? There is no clear answer today, but one may ask whether some or many of these patients do not fall within a category quite different from asthma as just defined—a category comprised of predominantly male patients in the older age group with relatively irreversible obstructive pulmonary disease, some of whom wheeze and some of whom do not, who tend to have respiratory infections repeatedly and who, as the process continues, are especially prone to develop respiratory acidosis and cor pulmonale. Some, but by no means all, have barrel chests and x-ray evidence of emphysematous blebs and bullae. Although this disease is sometimes referred to as "idiopathic," it is becoming increasingly clear that it is intimately associated with irritating dusts, smogs, fumes, and smokes. Among these patients, a history of heavy cigarette smoking is the rule. Just as asthma is defined here as an allergic bronchitis and bronchiolitis, these patients may well be regarded as having an irritative or inflammatory lesion in the same locations. The coalescence of air spaces and the development of blebs and bullae, often regarded as the primary lesion,

*Conference on Research in Emphysema, June 13-15, 1958.

may be merely a consequence of obstruction. If it becomes possible to weed out these instances of chronic obstructive pulmonary disease from among those belonging in the asthmatic category, the disease we call asthma may become a better-defined entity than it is today. This is certainly an objective worth striving for, and the allergist should share in the endeavor.

F. C. L.

American Academy of Allergy

ANNUAL REPORT OF THE COMMITTEE ON EDUCATION OF THE AMERICAN ACADEMY OF ALLERGY

THE annual meeting of the Committee on Education was held at the Bellevue Stratford Hotel in Philadelphia on Friday, Jan. 31, 1958.

The following were present:

Ben Z. Rappaport, Chairman
Carl Arbesman
George Blumstein
Charles Eyermann
William A. Howard
Robert G. Lovell
Kenneth P. Mathews
Max Samter
Paul M. Seebohm

The Committee reviewed the changes in the status of education in allergy during the past two years. The improved outlook was attributable largely to the establishment of the National Institute of Allergy and Infectious Diseases. In the fiscal year of 1956 (July 1, 1955, to June 30, 1956) the Institute supported one research grant in allergy ($4,293.00) and eight in immunology ($73,965.00). During the 1957 fiscal year the number of allergy grants was increased to seventy-one ($1,091,324.00) and the number of immunology grants to fifty-seven ($669,522.00). A further increase occurred in the 1958 fiscal year (July 1, 1957, to June 30, 1958) when ninety-two allergy grants ($1,339,584.00) and sixty-five immunology grants ($776,143.00) were made. In addition, there were in the fiscal year of 1958 twelve graduate training grants in allergy and immunology, totaling $254,145.00; one predoctorate fellowship for $3,600.00; and one special fellowship for $10,426.00. From the viewpoint of education, the research grants and the training fellowships are inseparable. The impressive numbers as well as the amount spent have produced an improvement in the education as well as research in allergy and immunology.

Report of the Committee on Postgraduate Education.—The work of this Committee, headed by Dr. Leo Criep, has continued to be outstanding. The large attendance of 350 at the various sessions is indicative of the success of the well-balanced program.

The Committee helped Dr. Ernest L. MacQuiddy to arrange a refresher course at the University of Nebraska.

Subcommittee on Undergraduate and Graduate Education (John Sheldon, Chairman).—Dr. Kenneth Mathews presented an interim report on a long-term project of testing graduating medical students for knowledge of allergy

in comparison with their knowledge of other medical subjects. So far the tests have been done in three medical schools. Further studies in many more schools with varying allergy teaching programs are planned before any conclusions can be drawn. It is hoped that by this study deficiencies in the teaching of allergy to medical students may be brought to the attention of the medical staffs of various schools.

Dr. Sheldon Cohen was encouraged to write a paper on his experiences in organizing a research group in allergy and immunology at Wilkes-Barre, a liberal arts college. The report, entitled "The Undergraduate Student and Scientific Research at a Liberal Arts College; An Experiment in Education," will appear in the *American Institute of Biological Sciences Bulletin.*

In response to a query from Dr. Carl Arbesman regarding the number of questions on allergy included in the examination of candidates for certification in pediatrics and internal medicine, the following information was obtained: The American Board of Internal Medicine replied that "2 to 3 per cent of the questions were pure allergy questions, and about 10 per cent of the questions were related to allergy, if one counts immune reactions, sensitivity, and hemolytic agents." The American Board of Pediatrics replied that in the past four years there were eight, three, four, and nine questions on allergy out of a total average of 171 questions.

Respectfully submitted,

Ben Z. Rappaport, Chairman.

Obituary

ROBERT B. SMITH

ROBERT B. SMITH died on Feb. 2, 1958. He was born in 1915 and received his degree in medicine from Ohio State University in 1940. From 1944 to 1946 he was a Fellow in internal medicine at the Henry Ford Hospital in Detroit. He became a member of the American Academy of Allergy in 1953. He was also a Fellow of the American College of Physicians, a member of the Akron Society for Internal Medicine, the Summit County Medical Society, the Ohio State Medical Association, and the American Medical Association. He had been chief of the Department of Medicine at St. Thomas Hospital and a member of the Senior Attending Staff of the City Hospital of Akron.

Announcements

Rocky Mountain Allergy Society

The following officers have been elected for 1958 by the Rocky Mountain Allergy Society:

President: Harold S. Tuft

Vice-President: John W. Bradley

Secretary-Treasurer: W. Grayburn Davis

Requests for 1959 Scientific Exhibits

Requests for space for scientific exhibits at the Chicago meeting, Feb. 9-11, 1959, are now being solicited. Application blanks may be obtained from the American Academy of Allergy Executive Office, 756 North Milwaukee St., Milwaukee 2, Wisconsin. If you know of any exhibits that you think we could attract, please forward the name of the principal exhibitor to the Executive Office and we will attempt to procure it.

George I. Blumstein, M.D., Chairman
Scientific Exhibits Committee